Consolidation of Human Memory

Brain Activations During the Retrieval of Word Pairs 1 Day and 1 Month Following Learning Measured with fMRI

Dissertation

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CONTENTS

ZUSAMMENFASSUNG

Gedächtniskonsolidierung umfasst alle Prozesse, die zu einer Stabilisierung der gelernten Information im Langzeitgedächtnis führen. Die funktionelle Magnetresonanztomografie (fMRT) ermöglicht es, Veränderungen in der Aktivierung bestimmter Hirnstrukturen während dem Erinnern konsolidierter Gedächtnisinhalte in gesunden Versuchsteilnehmern zu untersuchen.

Wir verglichen die mit fMRT gemessenen Hirnaktivitäten während dem Erinnern gelernter Wortpaare nach verschiedenen Konsolidierungszeiten, d.h. nach 24 Stunden und nach einem Monat.

Während dem Erinnern von 24 Stunden alter Information im Vergleich zum Erinnern von 10 Minuten alter Information fanden wir eine Zunahme der Aktivität im Bereich des linken Hippocampus, welche sich auch in einer Verstärkten Korrelation zwischen Erinnerungsleistung und fMRT-Signal im Hippocampus zeigte.

Nach einem Monat fanden wir wiederum eine Zunahme des Signals im Hippocampus sowie in neocorticalen Regionen, die für den Abruf von Wörtern von Bedeutung sind. Zudem sagte die Stärke des Signals im Hippocampus und Neocortex während des Tagesabrufs den Gedächtnisabfall über einen Monat voraus, d.h. je stärker das Signal nach einem Tag war, desto mehr Wortpaare wurden über den Monat vergessen. Die Aktivitätszunahme nach einem Monat ist Evidenz für die „multiple trace theory“, die besagt, dass Gedächtnisspuren während dem Konsolidierungs-prozess proliferieren und so zu einer besseren Integration der Information im Netzwerk führen. Alternativ kann die Aktivitätszunahme im Rahmen einer stärkeren Rekrutierung neuraler Strukturen bei Zunahme der kognitiven Anforderung interpretiert werden. Während sich schlechte Lerner bereits nach einem Tag stärker auf das Gedächtnisnetzwerk abstützen müssen, um die gleiche Leistung wie gute Lerner erbringen zu können, benötigen gute Lerner für eine genügende Erinnerungsleistung erst nach einem Monat mehr neurale Ressourcen.

Zusammenfassend zeigen unsere Resultate, dass die fMRT kurzzeitige Veränderungen, die vermutlich mit den synaptischen Veränderungen des Konsolidierungsprozesses im Zusammenhang stehen, bereits nach 24 Stunden abbilden kann. Die beobachtete Zunahme in hippocampalen und neocorticalen Arealen nach einem Monat kann im Rahmen einer stabileren Repräsentation der gelernten Inhalte im Gedächtnisnetzwerk interpretiert werden.

SUMMARY

Memory consolidation refers to the processes that follow a learning experience and lead to a certain stabilization of the information in long-term memory.

On one hand, the term is used for the molecular mechanisms that transform short-term memory into long-term memory and take place in the hippocampus within minutes to hours after learning. These transformation processes require gene transcription and protein synthesis and ultimately lead to the formation of new synaptic spines which are a potential for new synaptic connections.

In cognitive neuroscience on the other hand, the term consolidation is used for the changing contributions of different brain regions to memory storage and retrieval over time. The idea of a time-limited role of the hippocampus in memory retrieval has come from the observation of graded patterns of retrograde amnesia in patients with hippocampal lesions. Graded means that remote memories are preserved while recent memories are lost. According to the standard model, consolidation results in a shift from reliance of memory retrieval on both the hippocampal formation and the neocortex to the neocortex exclusively. Conversely, the multiple trace theory proposes that the hippocampus is continuously involved in the retrieval of episodic information. The formation of multiple memory traces over time and thus a stronger representation of remote memories are responsible for the graded pattern of retrograde amnesia. Studies on retrograde amnesia in human patients and lesion studies in animals suggest that these changes on a systems level need weeks, years or even decades to be completed.

Functional magnetic resonance imaging allows the investigation of brain areas that are associated with memory retrieval over time in healthy young participants. The few published neuroimaging studies on memory consolidation provide equivocal results. The reason might be the retrospective designs in which important variables such as learning procedure, rehearsal between study and test, and quantity and quality of retrieval could not be controlled. Moreover, most of the existing neuroimaging consolidation studies examined time periods of years or decades. Shorter time periods have rarely been investigated although recent neurobiological research has demonstrated that the formation of long-term memories is more dynamic than previously assumed. The fact that reactivation of a consolidated memory returns the memory into a labile state in which it can be strengthened, modified or erased

supports the hypothesis that changes in memory representation may be detectable with fMRI at shorter study-test intervals than only after years

We compared cerebral activity during the retrieval of word pairs after two different consolidation periods with a prospective design. First, we were interested in whether an fMRI signal enhancement in the hippocampus could be observed after the initial 24 hours, reflecting the molecular processes of consolidation. Next, we investigated whether further changes take place 1 month following this 24 hours period and whether the memory outcome after one month could be predicted by the hippocampal activity at the 1-day-lag. In both studies, we used incidental word learning to make sure that all participants processed the learning material similarly. Retrieval quality was assessed with the Remember/Know procedure and was equalized either empirically or statistically over the two time-lags.

The results of the first study revealed an enhanced left hippocampal activity during the retrieval after 24 hours versus 10 minutes. This increase was also expressed in an enhanced relationship between retrieval performance and the fMRI signal. The results demonstrate that short-term changes during the process of memory consolidation can be measured with fMRI. The results are also in agreement with findings in animals that suggest a strengthening of synapses in the hippocampus within 24 hours after learning.

In the second study, we found an increase in retrieval-related fMRI signal in the MTL and retrieval-associated neocortical regions after 1 month versus 1 day in good learners and the reversed pattern – MTL and neocortical increase after 1 day versus 1 month – in poor learners. Also, the strength of the fMRI signal in bilateral hippocampi at the 1-day-lag predicted the amount of forgotten word pairs after 1 month, i.e. the stronger the signal at the one-day-retrieval, the higher was the decrease in performance from the 1-day- to the 1-month-retrieval. Our data demonstrate that the hippocampal formation does not diminish its engagement in retrieval over 1 month of memory consolidation. A decrease can only be observed when there is a concurrent decrease in retrieval performance. The increase in fRMI signal over 1 month may be evidence for a proliferation and spreading of memory traces in the hippocampal formation as predicted by the MTT. According to the

correlation analysis the increase in activity might additionally result from an enhanced neural recruitment when cognitive demands are high.

In conclusion, our results reveal that fMRI is able to detect short-term changes that may be related to synaptic changes in the course of memory consolidation in time periods as short as 24 hours. The observed increase in hippocampal and neocortical areas 1 month versus 1 day after learning suggest that the MTL further intensifies its involvement in memory retrieval during the process of consolidation. This enhanced recruitment of MTL structures during the retrieval of remote memories may be evidence for a stronger representation of the learned material by multiple memory traces in these structures as predicted by the multiple trace theory.

ORIGINAL ARTICLES

The doctoral thesis is based on the following original articles:

A1 **Bosshardt, S.**, Schmidt, C. F., Jaermann, T., Degonda, N., Boesiger, P., Nitsch, R. M., Hock, C., and Henke, K. (in the press) Effects of memory consolidation on human hippocampal activity during retrieval.

A2 **Bosshardt, S.**, Degonda, N., Schmidt, C. F., Boesiger, P., Nitsch, R. M., Hock, C., and Henke, K. (manuscript in preparation) 1 month of memory consolidation enhances retrieval-related hippocampal activity.

My contribution to both articles was the planning of the design of the studies, assessment and analysis of behavioural and neuroimaging data (including the pilot study for the first study), and writing the first and second draft of the articles. In addition, I wrote the first drafts of the revision after the reviewing processes by journals the papers were submitted to.

Conny Schmidt and Thomas Jaermann of the Institute for Biomedical Engineering, University and ETH Zurich introduced me to the operation of the MR-scanner and supervised the measurement of imaging data. Nadia Degonda of the Division of Psychiatry Research, University of Zurich, helped me with the data analysis with the imaging software SPM and PMOD and discussed with me the designs of the studies and the interpretation of the results.

All work was done under supervision of Dr. K. Henke, Division of Psychiatry Research, University of Zurich.

Besides the two first authorship articles I also contributed to the following papers:

A3 Wollmer, M. A., Papassotiropoulos A., Streffer, J.R., Grimaldi, L. M. E., Kapaki, E., Salani, G., Paraskevas, G. P. Maddalena, A., de Quervain, D., Bieber, C., Umbricht, D., Lemke, U., **Bosshardt, S.**, Degonda, N., Henke, K., Hegi, T., Jung, H. H., Pasch, T., Hock, C., Nitsch, R. M. (2002) Genetic polymorphisms and cerebrospinal fluid levels of tissue inhibitor of metalloproteinases 1 in sporadic Alzheimer's disease. Psychiatric Genetics. 12 (3): 155-160.

A4 Degonda, N., **Bosshardt, S.**, Schmidt, C. F., Boesiger, P., Nitsch, R. M., Hock, C., and Henke, K. (submitted for publication) Implicit learning impacts explicit learning by way of the medial temporal lobe.

A5 Degonda, N., Schmidt, C. F., **Bosshardt, S.**, Boesiger, P., Nitsch, R. M., Hock, C., and Henke, K. (manuscript in preparation) Subliminal stimuli improve explicit learning in poor learners.

TALKS AND POSTERS AT SCIENTIFIC MEETINGS

Talks:

Meeting of the society for neuroscience, Orlando USA (2002): **Bosshardt, S.**, Schmidt, C. F., Jaermann, T., Degonda, N., Boesiger, P., Nitsch, R. M., Hock, C., and Henke, K. Effects of memory consolidation on human hippocampal activity during retrieval.

ZNZ Symposium, Zürich (2002) data blitz: **Bosshardt, S.**, Schmidt, C. F., Jaermann, T., Degonda, N., Boesiger, P., Nitsch, R. M., Hock, C., and Henke, K. Effects of memory consolidation on human hippocampal activity during retrieval.

ZNZ Ph.D Retreat, Valens (2001): **Bosshardt, S.**, Streffer, J., Nitsch, R. M., Hock, C., and Henke, K. Preclinical neuropsychological and neuroimaging markers for Alzheimer's disease. Presentation of the Ph.D. project.

Posters:

ZNZ Symposium, Zürich (2003): **Bosshardt, S.**, Degonda, N., Schmidt, C. F., Boesiger, P., Nitsch, R. M., Hock, C., and Henke, K. 1 month of memory consolidation enhances retrieval-related hippocampal activity.

ZNZ Symposium, Zürich (2002): **Bosshardt, S.**, Schmidt, C. F., Jaermann, T., Degonda, N., Boesiger, P., Nitsch, R. M., Hock, C., and Henke, K. Effects of memory consolidation on human hippocampal activity during retrieval.

ABBREVIATIONS

AD	Alzheimer's disease
BA	Brodmann area
BOLD	Blood-oxygen-level-dependent
CA	Cornu ammonis
cAMP	cyclic adenosine monophosphate
CREB	cAMP response element binding protein
ECS	Electroconvulsive shock
fMRI	Functional magnetic resonance imaging
fv-FTD	Frontal variant of frontotemporal dementia
MAPK	Mitogen-activated protein kinase
MTL	Medial temporal lobe
MTT	Multiple trace theory
NO	Nitric oxide
PET	Positron emission tomography
PKA	Protein kinase A
RA	Retrograde amnesia
REMO	Episodic retrieval mode
SD	Semantic dementia
SPECT	Single-photon emission tomography

1. INTRODUCTION

1. 1. Background

1.1.1. Episodic memory and its neural correlates

Memory consolidation is the term for the processes that occur after the acquisition of new information and that lead to a definitive and permanent storage of this information over time. For the understanding of consolidation theories, a basic knowledge about different memory systems is essential.

During the last century, the careful investigations of amnesic patients, i.e. patients who lost their memory have provided insight about where different kinds of information are stored in the human brain. The development of animal models on memory and selective lesions of specific brain regions in animals allowed building up a more detailed picture of which exact structures are needed for memory storage. And last but not least, the modern neuroimaging techniques PET and fMRI that experienced a surge in the last decade have further contributed to the investigation of memory processes by allowing the non-invasive study of learning and retrieval in healthy young participants. All methods together fostered the development of theoretical models of memory and the distinction between different memory systems.

One of the most important distinctions was suggested by Larry Squire (Squire, 1986; Squire and Zola-Morgan, 1991). The sources for the proposed model were the reports that described the memory deficits in patients with bilateral medial temporal lobe (MTL) damage, among which the patient H.M. (Scoville and Milner, 1957) is the most famous example. The medial temporal lobe (MTL) (Fig. 1) includes the hippocampal formation comprising the hippocampus proper (CA fields 1-4), the dentate gyrus, and the subiculum, the perirhinal cortex and the parahippocampal gyrus.

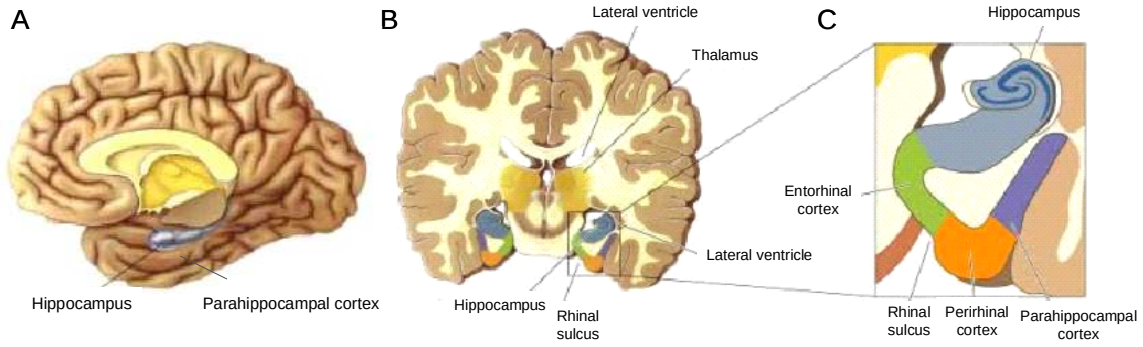

Fig. 1. The medial temporal lobe (MTL) in sagittal view (A), coronal view (B), and detailed view (C). Taken from the internet: http://www.physiology.wisc.edu/neuro524/learningI02/Slide12.JPG

Damage to the MTL resulted in a selective impairment of acquiring new memories (anterograde amnesia) and consciously retrieving memories formed before the onset of amnesia (retrograde amnesia, RA) demonstrating the vital role of this area for memory storage and retrieval. The fact that these patients were not impaired in performance of immediate memory, skill learning (Corkin, 1968) and other implicit forms of memory as for example priming led Squire to propose a model in which he firstly distinguished between short-term and long-term memory and within the domain of long-term memory further between declarative and non-declarative memory (Fig. 2).

Short-tem memory is capacity-limited and refers to information that can temporarily be kept in mind through ongoing rehearsal only (e.g. the 7 digits of a telephone number). Long-term memory on the other hand has no limited capacity, is long-lasting and cannot be disturbed by distraction because rehearsal is not necessary.

Declarative memory, as one form of long-term memory, is explicit and accessible to conscious awareness. It includes both episodic memory, which is memory for events with respect to a specific place and time, and semantic memory, which corresponds to our factual knowledge of the world. Declarative memory depends on the integrity of the neural systems that are damaged in amnesia, such as the MTL and diencephalic structures. Non-declarative memory, on the other hand, is implicit and accessible only through performance. It includes procedural learning, priming, classical conditioning, and other implicit forms of learning and memory. Non-declarative forms of memory rely on a variety of extra-MTL structures, such as the cerebellum, the

striatum, or the neocortex (Fig. 2). Both animal studies and the study of amnesic patients showed that declarative memory impairment becomes more severe as more components of the MTL memory systems including entorhinal and perirhinal cortices are damaged (Squire and Zola, 1996).

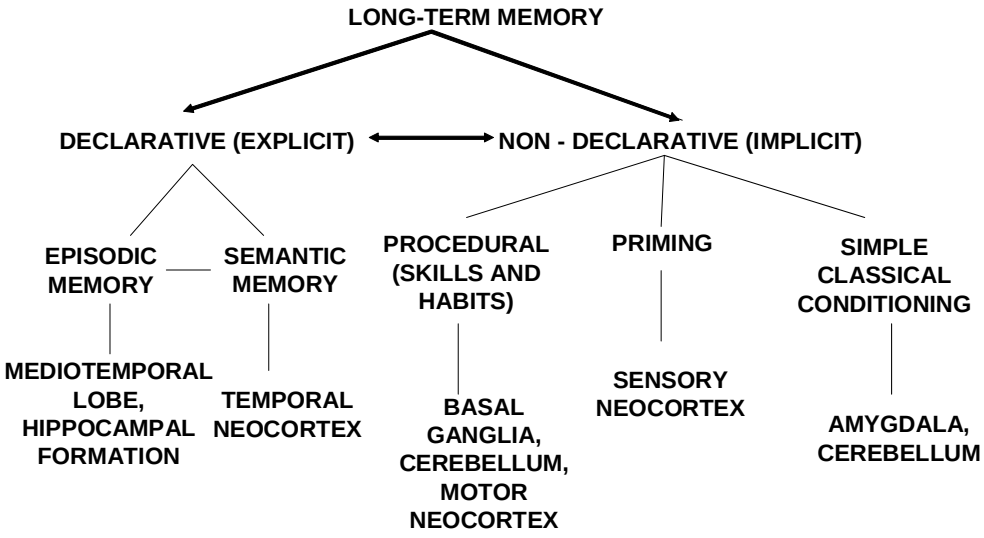

Fig. 2: The organisation of declarative and non-declarative long-term memory (adapted from Squire and Zola, 1996)

For the study of memory consolidation, the aforementioned concepts of episodic and semantic memory as further subcomponents of declarative memory has turned out to be crucial since, as will be outlined below, these two types of memory are believed to experience different fates during the process of memory consolidation (Nadel and Moscovitch, 1997). The distinction was proposed by Tulving (1972). Episodic memory stores information about personally experienced individual episodes or events, and the temporal-spatial relations among them. Essential is its specificity through its reference to space and time. Semantic memory contains organized factual knowledge about the world. This includes knowledge about words, their meanings and interrelations, and the rules how to use them (e.g. syntax and grammar rules). It also includes autobiographical facts, such as a former address, and other information that is probably acquired across many different contexts and which has no reference to a specific time anymore.

Since the 1990's it is well established that episodic memory depends crucially on the hippocampus while other forms of memory are associated with other structures (Baddeley, 2001). The precise role of the hippocampal formation and the MTL for episodic memory has further been refined by neuroimaging studies. It has been shown that the MTL memory system is associated with retrieval success but not with retrieval effort (Nyberg et al., 1996; Schacter et al., 1996). Attempts were also made to detect functional differences along the anterior - posterior axis of the hippocampus (Gabrieli et al., 1997; Strange et al., 1999; Small et al., 2001). Other studies have focussed on the precise mnemonic functions mediated by the hippocampus during encoding. Henke et al. (1997, 1999) demonstrated that the hippocampal formation is particularly important for establishing new semantic associations, a finding that supports the idea that the MTL is not the site of storage itself but that it provides associative links to the different neocortical regions representing distinct components of a remembered episode (Mayes and Roberts, 2001).

Regarding semantic memory, the picture is less clear. Whether both the retrieval and the acquisition of semantic memory (Squire and Zola, 1998) or the acquisition only (Cipolotti et al., 2001) or none of both (Vargha-Khadem et al., 1997) depend also on the integrity of the hippocampus is still a matter of debate. However, studies on patients suffering from semantic dementia have demonstrated that semantic memory primarily relies on neocortical areas of the temporal lobe, such as the inferior and lateral temporal gyri (Kopelman, 2002). These patients present with focal atrophy of the temporal neocortex and a relatively spared MTL and are strongly impaired in the retrieval of facts about the world but show almost normal performance in tasks of episodic memory.

Episodic and semantic memory are not only determined by the nature of stored information but differ also with regard to the form of consciousness they are associated with. A key feature of episodic memory is the possibility to mentally travel back in time, to re-experience a previous event at which one was present. This self-recollection during memory retrieval embodies a distinct form of subjective awareness, termed autonoetic (self-knowing) consciousness. Semantic retrieval on the other hand is detached from autobiographical reference. This noetic (knowing)

consciousness represents a status of knowing without self-recollection (Tulving, 1985; Gardiner, 2001).

So far, the distinction between episodic and semantic memory seems straightforward and clear. However, information about a personally experienced event can also be retrieved without autonoetically remembering or recollecting the event, but simply on the basis of their noetic knowledge or a feeling of familiarity. That I closed the door this morning when I left my flat can be retrieved just as a fact. I just know that I did so but cannot re-live the episode and remember any contextual details.

The Remember/Know paradigm (Tulving, 1985) accounts for this reality of episodic memories having a non-episodic quality. The Remember/Know paradigm requires participants in a memory recognition task to indicate whether they actually remember the occurrence of a presented item in the study list or whether they simply know that the item has been presented before. Tulving (1985) demonstrated that this distinction between Knowing and Remembering or familiarity and recollection, respectively is meaningful to people and that they can make corresponding judgements about their recognition of previous occurrences of particular events.

Given that episodic retrieval based on feelings of familiarity or knowing has a predominant semantic quality it is conceivable that it is mediated by neural substrates that are close to those representing semantic memory.

Indeed, recent imaging studies (Henson et al., 1999; Eldridge et al., 2000; Davachi et al., 2003) and one study on patients with different temporal lobe pathologies (Yonelinas et al., 2002) support the hypothesis that Remembering and Knowing an event have different neural substrates. It has been shown that memories based on recollection are associated with the hippocampus whereas the surrounding temporal lobe including the perirhinal and parahippocampal gyri is believed to support memories based on familiarity. Since recognition can be performed solely on the basis of feelings of familiarity, these studies support also the hypothesis of Aggleton and Brown (1999) that recognition memory relies on the integrity of the perirhinal cortex but not the hippocampus. They assume that the hippocampal-anterior thalamic system supports recollection-based recognition (Remembering) whereas cortical (perirhinal) systems support familiarity-based recognition (Knowing) of episodes. Thus, very selective hippocampal system damage may spare those tests of recognition memory that can be performed effectively by using just familiarity.

This chapter has shown that although not undisputed (Squire and Zola, 1998; Manns et al., 2003), declarative memory is not a unified entity but can be divided into qualitatively different components according to differences in the nature of the stored material and the type of consciousness associated with the retrieval. Moreover these components of declarative memory appear to rely on different neural substrates such as the hippocampus for true episodic memory with autonoetic consciousness, the perirhinal and parahippocampal cortices for semantic retrieval of episodes with noetic consciousness and the adjacent neocortical temporal cortices for true semantic memory, i.e. knowledge.

The differentiation between distinct components of declarative long-term memory and their reliance on different neural regions is important for the understanding of the systems consolidation theories, especially the multiple trace theory, which are presented in the next two chapters.

1.1.2. Systems consolidation theories

1.1.2.1 The standard model

Graded patterns of retrograde amnesias (RA) in patients with brain injuries have been observed and described since the late 19th century. Graded means that amnesia is more severe for events that happened shortly before the trauma than for events that extended long time back to the past. In 1882, Ribot expressed this phenomenon in his “Loi de Regression” which made him one of the founders of the consolidation theory (for a review see Sara, 2000 and Kopelman, 2002).

In the past three decades, several studies on patients with hippocampal lesions confirmed the graded nature of RA, in which remote memories were retrieved better than recent memories. The widely accepted interpretation for the sparing of remote memories after hippocampal damage is that the retrieval of information is dependent on the hippocampus only for a limited time. As time passes, a gradual reorganisation takes place whereon neocortical sites gain the control over memory. This process of reorganisation by which memory becomes independent of the hippocampal formation is termed consolidation. Spatial reorganisation must not necessarily mean that

memory stores are transformed from the hippocampus to neocortical sites. The neural network model of consolidation suggests that medial temporal structures bind together the multiple neocortical sites that store the memory representation and that after repeated reactivation (consolidation) the cortico-cortical connections become strong enough to support memory retrieval without the medial temporal lobe (see Squire and Alvarez, 1995).

However, studies with patients always rely on retrospective assessment of memory. This has the disadvantage that the comparison of performance levels of remote and recent memories is not very reliable. Only prospective studies may provide control over timing and strength of original learning (Murray and Bussey, 2001). Such prospective studies in animals which started being conducted in the 1990s have also the advantage that lesion size and location can more reliably be controlled. To date, a number of prospective animal studies with a wide variety of memory tasks and in different species have replicated the findings of graded patterns of RA (Zola-Morgan and Squire, 1990; Kim and Fanselow, 1992; Anagnostaras et al., 1999; Clark et al., 2002). The extent of observed RA is variable and extends from about 5 days to 30 days.

In their reviews of studies on RA in patients (Squire and Alvarez, 1995) and in animals (Squire et al., 2001) Squire and his co-workers find that damage limited to the CA1 fields of the hippocampus causes a limited RA whereas damage extending to adjacent parahippocampal regions causes extensive RA. RA in humans varies between several months to decades whereas in animals the extents are much shorter and lie within the range of days. However, they cite also studies, in which the patients described exhibited no graded pattern, but a flat gradient of RA, meaning that these patients were equally disturbed in the retrieval of recent and remote memories. The standard model of consolidation proposes that patients with a flat gradient must have pathology extending to adjacent cortex or even beyond the medial temporal lobes. The theory cannot account for flat gradients in patients with circumscribed damage to the hippocampal formation. Also, the theory does not differentiate between episodic and semantic memories. These weaknesses encouraged critics of the model to think of alternative explanations for the different patterns of RA.

1.1.2.2 The multiple trace theory

In their review of RA literature, Nadel and Moscovitch (1997) come like Squire et al. (1995) to the conclusion that the extent of RA, and whether or not it is graded, depends on the amount of medial temporal damage. But unlike the proponents of the standard model, they find clear dissociations within RA among different types of memory. Memory for autobiographical episodes appears to be most severely affected by MTL lesions, followed by memory for personal semantics, public events and persons. Least affected seems to be general semantics including conceptual knowledge of words, grammar and objects. RA for autobiographical events extends back to almost the entire lifetime with either absent or very shallow gradients. Gradients for personal semantics and public events and persons range from 10 to 30 years. Corresponding results are found in the review of animal literature. For tasks for which the hippocampus is absolutely essential, a flat RA gradient is found, for other tasks, the typical findings are either no RA at all or a graded RA.

According to Nadel and Moscovitch (1997), the standard model cannot sufficiently explain the reported data, especially not the very extensive and often flat RA gradients in autobiographical memory or the evidence of relatively preserved semantics which were acquired during periods for which subjects have RA. Moreover, they question the adaptive basis for a consolidation process that is almost as long as the average human life span. Instead, they provide a biologically and psychologically plausible model that should better account for the existing evidence and that does not rely on the assumption of a long-term consolidation process during which the retrieval of information becomes independent of the hippocampus and subsequently relies entirely on the neocortex. The model is termed the multiple trace theory (MTT) and proposes that memory traces consist of neocortical representations, bound by the hippocampus, that mediate the experience of an episode. Over time and upon repeated reactivation, multiple traces are formed that share some or all of the information about the initial episode. Only facts that can be extracted from context eventually become independent from the episodic memory trace. The spatial and temporal contextual information that conveys the episodic quality to memory depend continuously on the hippocampus. Thus, as episodic memories age, they would either be forgotten or would be represented as multiple traces in a hippocampal-neocortical network. This multiple representation of older

memories renders them less vulnerable to hippocampal damage. The disruption of old memories would require an extensive lesion of the hippocampus whereas newly acquired memories would be affected by minimal damage already. Semantic memories are ultimately stored independently of the hippocampal complex and are thus least vulnerable to disruption. The model corresponds well to the reported evidence on RA, in that the extent and gradient of the RA is determined by the episodic richness of the trace that is to be recovered on one hand and the extent of the lesion on the other hand.

The model is also supported by recent studies on autobiographical memory in patients with MTL damage. Cipolotti et al. (2001) present a case with selective hippocampal pathology and flat extensive RA. Interestingly, this patient also exhibits deficits in acquisition of new semantic knowledge indicating that the hippocampus plays a role in the initial storage of both episodic and semantic information. Piolino et al. (2003) compared the patterns of amnesia in dementia patients with MTL pathology (early Alzheimer's disease, AD), neocortical pathology in the anterior temporal lobe (semantic dementia, SD), and the frontal lobe (frontal variant of frontotemporal dementia, fv-FTD). They found that in AD and fv-FTD patients most strictly episodic memories (i.e. unique, specific in time and space, detailed) from all periods of life were equally impaired supporting the hypothesis of a continuous involvement of the hippocampus in retrieval. In SD patients a reversed temporally graded pattern for autobiographical memories was observed. This reversed pattern with a worse retrieval of remote episodic information was interpreted in terms of an increasing importance of the personal semantic component for reconstructing autobiographical memory over time. They also conclude that the often reported preserved remote memories in AD patients and presumably other patients with amnesia must have a predominantly semantic character.

These studies draw attention to the interactions between semantic and episodic memory. Personal experience supports the maintenance of semantic knowledge whereas semantic memory in turn provides an organizational structure that guides the retrieval of episodes (Hodges and McCarthy, 1995). Amnesia for semantic memories in patients with lesions to the episodic memory system in the MTL occurs when the memories are still contextually bound and not yet fully integrated into the semantic (i.e. neocortically represented) system (Rosenbaum et al., 2001) or, as put by Westmacott et al. (2003), when the semantic knowledge is autobiographically

significant. Autobiographically significant knowledge refers to semantic concepts that are more likely than others to be associated with specific personal memories. Westmacott et al. (2003) emphasize the need for memory tests that can differentiate between autobiographical and semantic components of a memory representation, because it is possible that each aspect of memory is differentially affected by memory age and by damage to different brain regions.

This dynamic and interactive nature of memory was also put forward by the promoters of the MTT Nadel and Moscovitch (1997, 2001) and Nadel and Land (2000). The nature of the memory may change with time. Every reactivation modifies a given memory by integrating it into the context of reactivation. Importantly, memory is not a unitary entity, but different attributes may experience different fates. The essence of an experience is more likely to be reactivated than details. Therefore, multiple traces are often formed for the core elements of an episode whereas contextual details may get forgotten. The repeated reactivation may allow for abstraction of the essentials of an event and the memory may become fact-like and thus independent of the MTL.

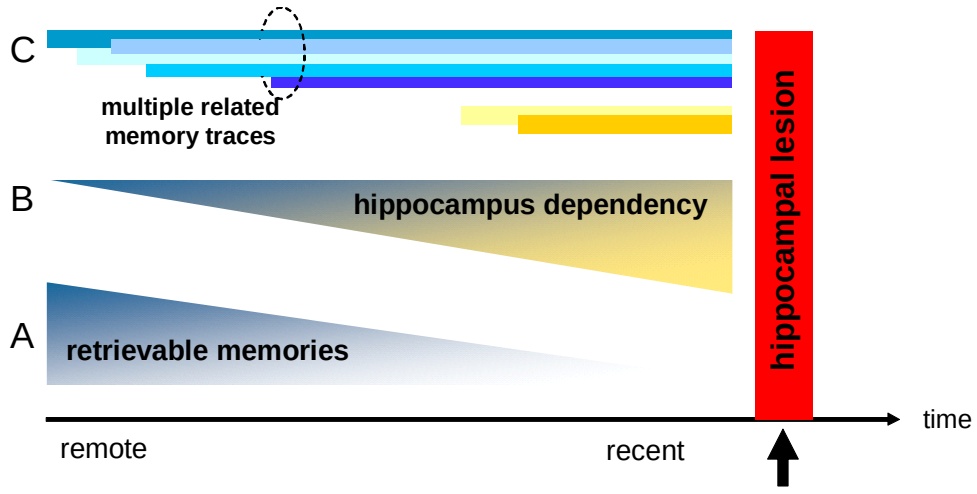

Fig. 3. The two models of systems consolidation that explain the temporally graded pattern of retrograde amnesia (A): The standard model (B) suggests that only recent memories are dependent on hippocampal function whereas remote memories rely on neocortical structures. According to the multiple trace theory (C), multiple related memory traces are formed over time. This proliferation makes remote memories less sensitive to disruption by hippocampal lesions.

The standard model and the multiple trace theory both support the idea that over time memories become more resistant to lesions to the hippocampal formation. Whereas in the standard model this resistance is believed to be achieved by a shift from reliance on both the hippocampus and the neocortex to the neocortex exclusively, the multiple trace theory proposes that multiple related traces are formed within the hippocampal complex and as a result render memories less vulnerable to MTL lesions. In both models, the extent of the lesion determines the extent and gradient of the RA. Importantly, the multiple trace theory emphasizes also the influence of the memory type on the pattern of RA. The more episodic a memory, the more it depends on the hippocampal formation (Nadel et al., 2000). In contrast, the standard view supports the idea that fact and event-specific information are equally dependent on the hippocampal formation initially and over time.

1.1.3 Neuroimaging evidence

Clinical studies bear the disadvantage that lesions of brain damaged patients are difficult to specify. Fully complete and fully selective hippocampal lesions were hardly observed (Eichenbaum, 2001). Furthermore, clinical studies are necessarily retrospective. In animal studies, where a prospective approach is possible and the lesions can be determined with much greater precision, the differentiation between semantic and episodic memory is not easy to model. An additional approach to lesion studies is functional neuroimaging. It allows comparing whether the same or different brain areas are active during the recollection of remote versus recent information. The standard model would predict a temporal gradient in hippocampal activity with remote memories provoking no MTL activation or a decrease in activation when compared to recent memories. According to the MTT on the other hand, no differences in hippocampal activation would be expected as long as the episodic quality of recent and remote memories is equal.

The few neuroimaging studies published so far provide equivocal results. Maguire (2001) reviewed literature from 1995 to 2001 on autobiographical long-term memory and found overall left lateralized medial frontal and hippocampal activations for autobiographical event memory. For semantic remote memory, no MTL activations

were reported. Although in most studies there was no direct comparison between recent and remote memories, these left hippocampal activations imply that for autobiographical event memory, unlike for semantic memory, no complete reorganization had taken place. Also in a direct comparison between recent and remote memory, Maguire et al. (2001) found a modulation of hippocampal activity in relation to memory type but not to memory age. Similarly, Ryan et al. (2001) found equivalent levels in hippocampal activation during the retrieval of 4 years old versus 20 years old event memories. However, Haist (2001) found temporally graded changes in entorhinal cortex during retrieval of famous faces from different decades. Niki and Luo (2002) investigated the retrieval-associated brain activations during recall of places visited recently or seven years ago and reported increased left parahippocampal activity when recent memories were contrasted with remote memories. Piefke et al. (2003) observed increased activity in recent versus remote cued recall of autobiographical events in bilateral hippocampi and retrosplenial cortices. A less clear pattern was reported by Tsukiura et al. (2002). They found an increase in parahippocampal activations during the cued recall of episodes from childhood and a recent periode but not from adolescence. And last but not least, Maguire and Frith (2003) observed a lateral asymmetry in the hippocampal response to the remoteness of autobiographical memories. The right hippocampus showed a temporal gradient, decreasing in activity the more remote memories were whereas the left hippocampus showed no relation to the age of memories.

All together, the present data neither speak clearly in favour of the standard model nor of the multiple trace theory. The reason for these inconsistent findings as well as important methodological consequences for the comparison of recent and remote retrieval in neuroimaging studies will be discussed in the next section.

1.1.4 Methodological issues for the neuroimaging of consolidation

The conflicting results between the reviewed studies can be explained by different methodological approaches. A general problem of all cited studies is that they are retrospective.
In retrospective studies there is no objective information about the encoding episode. What was the context during encoding? How well and how fast was the information acquired? Different learning circumstances during recent and remote learning could influence the storage and subsequent retrieval (Murray and Bussey, 2001).

An additional problem in retrospective studies is the reactivation and rehearsal of the learned material. Episodes from the past are probably more often reactivated than recent episodes. According to the dynamic nature of memories, remote memories are therefore likely to become more fact-like and lack the richness of details that characterize recent memories. In addition to the lack of control over spontaneous reactivation between the learning episode and the retrieval session, all studies except those by Haist (2001) and Tsukiura et al. (2002) had the weakness that participants had to reactivate their memories during an assessment of details of autobiographical memories in semi-structured interviews some days or weeks prior to scanning. Consequently, it is not clear whether they scanned the recall of the interview session or the original event. Moreover, as outlined above the nature of those memories might have changed as a consequence of the questioning such that the recall of these memories is qualitatively different from the recall of the original event. This hypothesis of a change of memory traces after reactivation is also supported by the neurobiological findings on reconsolidation (see 1.1.5).

No study did assess the amount and quality of retrieval directly during scanning. Mostly, only post-scanning evaluations were used to provide information about what was retrieved during the measurement of brain activity. However, it is important to know the behavioural correlates associated with the measured brain activities. Otherwise, activity differences might simply reflect differences in retrieval quantity or quality and not effects of consolidation. Ideally, retrieval performance is equal for both retrieval delays. Free recall is difficult to assess in neuroimaging due to speech-related movement artefacts. Therefore, memory performance is commonly assessed

with cued recall or recognition tasks. Quality of retrieval can be assessed with the Remember/Know paradigm (Tulving, 1985).

Some studies (e.g. Haist, 2001; Niki and Luo, 2002) did not at all differentiate between different types of memory, while other studies (e.g. Maguire and Frith, 2003; Maguire et al., 2001; Piefke et al., 2003; Tsukiura et al., 2002) aimed to measure strictly autobiographical event memory. Ryan et al. (2001), Piefke et al. (2003) and Maguire and Frith (2003) also controlled for emotional value of the episodes, which was not done by other groups. On grounds of the theoretical dissociation of different memory types regarding consolidation and long term storage (Nadel and Moscovitch, 1997) and the activation differences found between autobiographical event and autobiographical fact memories (Maguire, 2001; Maguire *et al*., 2001) it seems crucial to control for true episodic retrieval over the measured time lags. Further, the control of emotional value and the degree of personal relevance of memory seems to be reasonable too.

Only a prospective design can meet all the criteria that allow a reliable interpretation of activity differences between recent and remote memories. So far, only one study by Stark and Squire (2000) tackled the problem with a prospective design. They compared memory associated MTL activities during retrieval of line drawings one-half hour, 1 day and one week after learning. Although they did equalize performance levels across the three delays, no effect of study-test interval was observed. The authors concluded that the time span was too short to observe changes in activity.

1.1.5 Cellular consolidation and reconsolidation

In the previous sections, consolidation was discussed from a systems perspective only, which means that at the core of interest was the role of the hippocampus and its interaction with other cortical regions in the formation of long-term memories. In cellular neurobiology however, consolidation refers to the cellular and molecular processes that take place within minutes to hours after learning within the hippocampus. Common to both the systems and the cellular view of consolidation is that training or learning starts a cascade of transformation processes that convert

memory from a labile into a stable state of representation. During the labile state, memory representations are sensitive to disruption by various treatments inducing amnesia.

Whereas in systems consolidation the mechanisms of transformation remain controversial, evidence for the processes that underlie the cellular consolidation is more convergent. The transformation from short-term to long-term memory involves activation of gene expression, new protein synthesis and the formation of new synaptic connections in the hippocampal formation (Milner et al., 1998). The core signalling pathway for this transformation is the activation of the enzymes protein kinase A (PKA) and mitogen-activated protein kinase (MAPK) by increase of the second messenger cyclic AMP (cAMP) in the presynaptic terminals. These enzymes phosphorylate and activate the cAMP response element-binding (CREB) protein in the nucleus. CREB in turn activates several genes leading to persistent activity of PKA, to protein synthesis and to the formation of new dendritic spines (Kandel, 2001). It has been shown that spatial training in rats results in an increase of synaptic spines in the dentate gyrus within 6 hours post-training (O'Malley et al., 2000). A very recent study even demonstrated that an increase in spine density in the hippocampal CA1 field 24 hours after training was specific to learning a positive association between two conditioning stimuli (Leuner et al., 2003). These data indicate that associative memory formation is accompanied by an increase in spine density in the hippocampus which may ultimately result in the development of new synaptic contacts.

The traditional view of cellular consolidation is that when this learning-induced signalling pathway is interrupted at any part (e.g. by protein synthesis inhibitors or electroconvulsive shock (ECS) treatment), the memory trace cannot be stabilized which results in amnesia for the learned episode. Importantly, these treatments induce amnesia only when given within a sensitive time period after learning. In behavioural studies with animals, this sensitive time period usually lasts a few hours after which the memory representations are resistant to interference (Dudai, 2002).

The idea of a clear distinction between a labile short-term memory and a stable or fixed long-term memory was challenged by the findings that previously resistant memories could be rendered vulnerable to disruption again by presenting a training cue immediately before the disrupting intervention (Misanin et al., 1968; Nader et al.,

2000a; Pedreira and Maldonado, 2003). The presentation of appropriate training cues is thought to reactivate the memory trace, and the processes that follow to convert it into a stable representation again are termed reconsolidation. The same manipulations that cause memory loss after initial learning can also destroy the trace during reconsolidation. Again, amnesia can only be induced when the manipulation is administered during a limited time-window. The phenomenon of reconsolidation has been demonstrated in a variety of species and across a large array of memory paradigms. There is evidence that during reconsolidation the same molecular cascade activated by new learning and formation of long term storage, including NMDA receptor activation, CREB-mediated transcription and new protein synthesis, could be recapitulated (Nader, 2003). An important difference between consolidation and reconsolidation appears to be the time needed to re-stabilize a memory trace. A consistent finding across paradigms is that reconsolidation occurs faster than consolidation, indicating that during reactivation no complete reversal of the initial morphological changes that followed the first learning experience occurs. As for consolidation, parametric designs that vary the time between learning or reactivation and consolidation or reconsolidation are necessary to assess the time-related characteristics of the mechanisms.

Recently, Debiec et al. (2002) have found evidence that reconsolidation occurs also at the systems level. When memories, that were 45 days after contextual fear conditioning not anymore sensitive to hippocampal lesions, were reactivated immediately before the lesion, amnesia was observed after the lesion. They concluded that reactivation of a remote memory would cause it to return to a labile hippocampus-dependent state. Interestingly, the labile state during reconsolidation lasted far shorter (1-2 days) than the initial consolidation period.

Nadel and Land (2000) have proposed that repeated reactivation and reconsolidation over the long term contributes to the formation of multiple memory traces (see MTT 1.1.2.2) by a selective strengthening of specific attributes which in turn make memory as a whole more retrievable. Nader et al. (2000b) objected that reconsolidation will not induce the formation of new memory traces. Instead, they propose that it is the old trace that undergoes reconsolidation. Indeed, there is no consensus, whether reconsolidation replaces a previously stable representation or whether newly established representations are added and eventually coexist with the old trace (Miller and Matzel, 2000).

If retrieval is regarded as a result of the integration of incoming environmental information with the memory network driven by that information as proposed by Tulving and Thompson (1973), it follows that each reactivation will change the memory content because the situations during which reactivations may occur are always different. From this point of view, it seems conceivable that stable traces must be weakened during reactivation to allow a modification of the trace or an interweaving with new information and thus an incorporation of new traces.

It is important to note that reactivation of a given memory occurs not only when appropriate training cues are explicitly presented. Rather, reactivation is suggested to be part of the consolidation process leading to an integration of the new memory into the brain's long term storage. Evidence for this hypothesis comes from the findings that patterns of neural activity in hippocampus and neocortex, expressed during a behavioural episode, can be spontaneously re-expressed during subsequent 'off-line' states, such as slow wave sleep and quiet wakefulness (Sutherland and McNaughton, 2000). Although this spontaneous implicit reactivation is reported to be most frequent in the first hours after the learning episode, there is evidence for occasional reactivation at least 24 hours after the experience. It appears that reactivation on one hand supports the primary stabilization of information and on the other hand triggers the mechanism of reconsolidation and by way of this guarantees the permanent updating and change of stored memories.
In conclusion, research on reconsolidation has demonstrated that the mechanisms that underlie memory are far more dynamic than it was assumed before.

As outlined in the previous sections, research on systems consolidation and cellular consolidation differ in many aspects. Whereas systems consolidation is primarily interested in the contribution of different brain regions to memory storage and retrieval over time, research on cellular consolidation tries to elucidate the cellular and molecular mechanisms that start with a learning experience. Accordingly, methods of research in the two fields are completely different. Systems consolidation is either investigated by assessing encoding and retrieval in patients with hippocampal lesions or lesioned animals or by measuring retrieval-associated brain activations in healthy humans with neuroimaging procedures like PET and fMRI. Cellular consolidation research focuses on neurobiological methods in animal

models. Further, the investigated time-period covers minutes to days in cellular research versus years to even decades in systems consolidation in humans.
Whereas attempts are being made to find cellular and molecular correlates of systems consolidation (Shimizu et al., 2000; Frankland et al., 2001), the short-term changes that occur within the first days or months after learning have hardly been investigated with neuroimaging methods in humans (for an exception see Stark and Squire, 2000).
However, it is bio - logically not plausible that a memory trace is stored in the hippocampus and waits there unmodified until it will get consolidated after years or even decades, as suggested by the findings of temporal gradients of RA and the traditional consolidation theory. Instead, it can be assumed that memory traces are being strengthened, modified or eliminated (forgotten). Neuroimaging offers the opportunity to investigate these dynamic processes in healthy humans.

2.2 Methods: functional magnetic resonance imaging

The introduction of functional magnetic resonance imaging (fMRI) which lies back little more than a decade has revolutionised research in cognitive neuroscience. With its development came the possibility to not only look non-invasively at the anatomy of the brain within the living human but also to assess cognitive functions and map them to the brain at the same time. Fig. 4 shows a typical experimental set up of an fMRI experiment.
FMRI has become the method of choice for many functional activation studies in humans because of the following advantages: First, it is non-invasive without any risks of irradiation hazards, thus allowing repeated use in the same volunteers. Second, it provides both anatomical and functional information in each subject at the same session. Third, it has better spatial and temporal resolution than other methods that are used to localize neural activity (i.e. PET, SPECT) (Kollias, 2002).

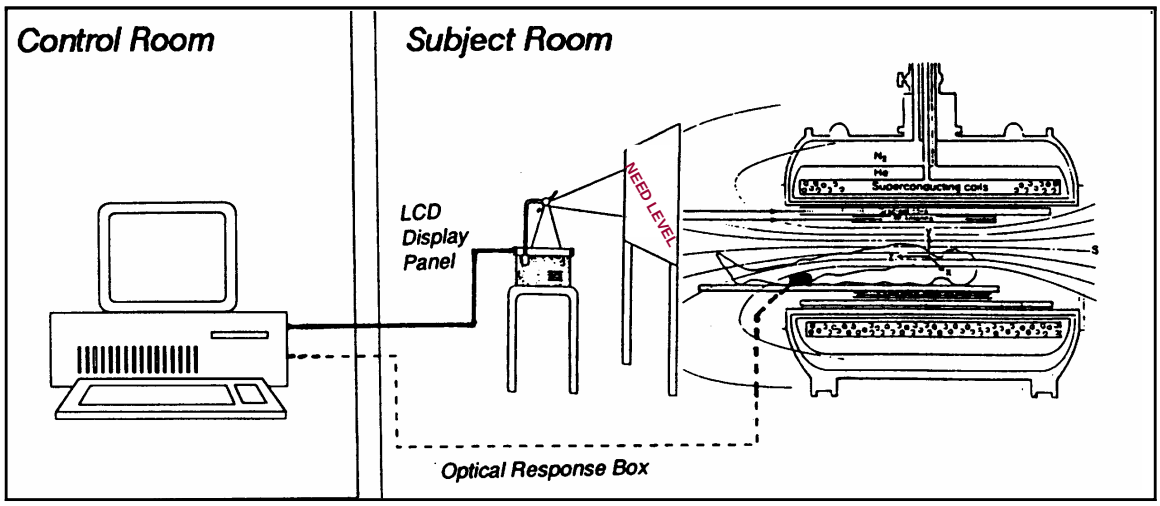

Fig. 4. Experimental set up of an fMRI examination. The participant is placed in the MR-scanner and can communicate with the experimenter in the control room by a two-way intercom system. The task is displayed via LCD projector to the back of a screen the participant watches through a mirror fixated to the head coil. Answers are given by button press via an optical response box that is connected by a glass fibre cable with the computer in the control room.

1.2.1 The neural basis of the functional magnetic resonance signal

FMRI is not a direct measure of neural activity. Instead, signal intensity alterations are caused by stimulus-induced local changes in blood oxygenation. This phenomenon is termed the BOLD (blood oxygen-level-dependent) contrast (Ogawa et al., 1992). The physical basis of the BOLD contrast is the oxygenation-dependent magnetic susceptibility of haemoglobin. Deoxyhaemoglobin is paramagnetic and acts as an intrinsic contrast agent. It leads to inhomogeneity in the magnetic field surrounding the vessels and therefore reduces the MR image intensity via an increase of T2* relaxation rates which are sensitive to field inhomogeneity.

Neural activity is accompanied by an increase in glucose consumption and blood flow that far exceed the increase in oxygen consumption (Fox et al., 1988). This overcompensation of oxygenated blood reduces the total paramagnetic blood deoxyhaemoglobin content causing the MRI signal to increase. The hemodynamic response has a characteristic time course. After stimulus onset, the signal increase is

delayed by 2-3 s, followed by a ramp of 6 – 12 s to a plateau or peak value returning then with a similar ramp and an undershoot back to baseline (Fig. 5a) (Kwong et al., 1992; Boynton et al., 1996).

Long time the biological mechanisms that underlie the relationship between these localized increases in BOLD contrast and neuronal activity remained unclear. Only research during the past few years has shed light onto both the cellular mechanisms of metabolic and vascular processes that are relevant to functional imaging and the neurophysiological underpinnings of the fMRI signal.

The basic principle of functional neuroimaging methods like fMRI and PET is that neuronal activity is tightly coupled to changes in blood flow and oxygenation and energy metabolism. Recent data suggest that both, the vascular and the metabolic response to neuronal activity are mediated by glutamatergic neurotransmission (Magistretti and Pellerin, 1999; Attwell and Iadecola, 2002; Bonvento et al., 2002). The amino acid glutamate is the main excitatory neurotransmitter in the brain and is released following neuronal activation. After it is released, glutamate needs to be removed promptly from the synapse and this occurs by uptake into an adjacent non-neuronal cell – an astrocyte. There it is converted to glutamine before it can be returned to the neuron where it is recycled. The neurometabolic coupling to glutamatergic neural activity is based on the fact that all steps from the release of glutamate to its transformation as well as its actions on postsynaptic receptors require energy (mostly due to the necessary re-establishment of ion gradients). This energy demand is preferentially met by the glycolytical transformation of glucose into lactate in astrocytes and by oxidative phosphorylation of glucose and lactate in the neuron. Both pathways contribute significantly to glucose consumption.
Neurovascular coupling to glutamatergic neural activity is explained by the release of nitric oxide (NO) and prostaglandins from neurons or astrocytes upon a glutamate invoked intracellular rise in Ca2+. NO and prostaglandins are potent vasodilator agents and thus leading to an increase in blood flow during activation. Although, neurometabolic and neurovascular coupling are both mediated by glutamatergic transmission, the increase in blood flow and energy utilization can be dissociated and should be considered the results of processes operating in parallel. This view

represents a shift from the traditional idea that the BOLD response is determined by the energy demands of the tissue directly.

The neurophysiological basis that underlies the BOLD signal has been investigated by simultaneously recording neuronal activity and fMRI. A number of studies have demonstrated that the hemodynamic response reflects directly an increase in neural activity elicited by a stimulus (e.g. Ogawa et al., 2000; Rees et al., 2000). BOLD responses and neural responses even seem to have a linear relationship for stimulus presentations of short duration.
With their electrical recording techniques in monkey primary visual cortex Logothetis et al. (2001) were even able to distinguish between spiking activity and local field potentials. Spiking activity represents the output firing of individual neurons and groups of neurons whereas local field potentials represent cortical input and local intracortical processing. They found that changes in the local field potentials are more closely related to the evolution of the BOLD signal than changes in spiking activity suggesting that the fMRI signal mainly reflects the incoming specific or association inputs into an area and the processing of this input information by the local cortical circuitry. Very often the incoming subcortical or cortical input to an area will generate output activity. In this case, the spike rate will indeed be correlated to the measured BOLD signal (Logothetis, 2003).

For a more detailed understanding of the neural basis of the BOLD signal, the following articles can be highly recommended (Magistretti and Pellerin, 1999; Bandettini and Ungerleider, 2001; Raichle, 2001; Bonvento et al., 2002; Lauritzen and Gold, 2003; Logothetis, 2003;).

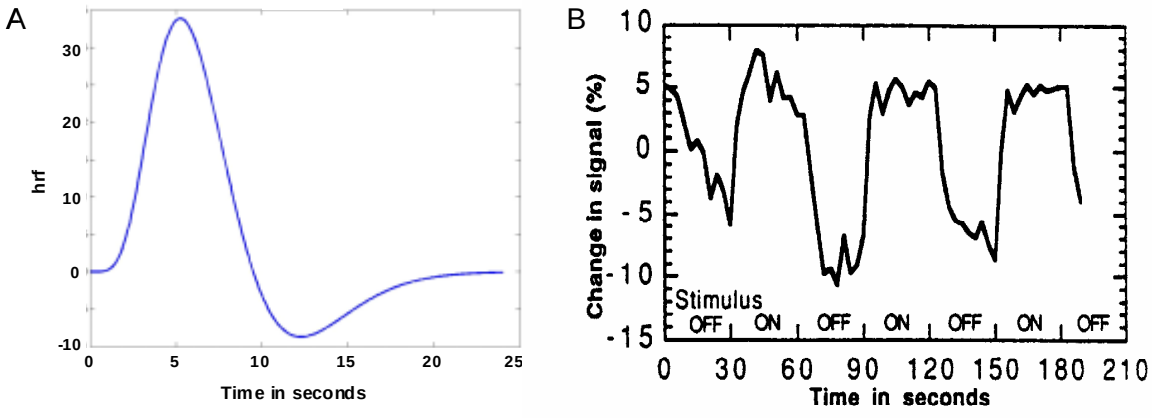

Fig. 5. The hemodynamic response function following a single stimulus (A) and the signal change in a blocked design (B). ON stands for the experimental condition, OFF for the baseline condition. Pictures taken from www.cmrr.umn.edu/ ~eja/Images/bold.gif

1.2.2 The baseline task, cognitive subtraction and cognitive conjunction

The signal increase elicited by a cognitive task is typically not measured absolutely in fMRI but relative to a baseline condition (Fig 5b). Differences between brain activity during task and baseline are then attributed to the mental processes elicited by the task. Traditionally, such a baseline was established by using periods of rest. Because in such rest periods no task is performed, it was assumed that they represent something like a resting brain that can be compared to an active brain during cognitive challenge. However, several recent studies have demonstrated that the conscious resting state is not a state of neural inactivity, and that activity during rest may even be greater than in some cognitive tasks (Stark and Squire, 2001). In the absence of external information and without explicit task instructions one experiences a stream of thoughts consisting of mental images, verbal ideas and other phenomena. Binder et al. (1999) have shown that the cognitive processes behind this day dreaming are semantic retrieval, representation, and information manipulation. This state can be expected to occur also during states similar to rest, such as fixation and passive presentation. When subtracted from the experimental task, the use of such a resting baseline would disguise areas involved in semantic retrieval and episodic encoding and manipulation and is therefore a non-optimal choice for most cognitive tasks.

More appropriate baseline conditions for cognitive fMRI studies are tasks where the mental processes and associated brain activations that are induced by the task are known. The main constraint for an optimal baseline task is that it engages in the same mental operations as the experimental task except for the process of interest. In the cognitive subtraction design (Fig. 6a) (Friston, 1997) the common processes of experimental and baseline task are eliminated and activity differences reflect the processes of interest.

A more sophisticated approach to analysing cognitive activation is the cognitive conjunction method (Fig. 6b). While in cognitive subtraction the **two tasks** (task and baseline) of a pair differ only by the process of interest, cognitive conjunction experiments are designed such that **two or more task-baseline subtraction pairs** share a common process of interest (Price and Friston, 1997). Cognitive conjunction analysis reveals brain areas that are commonly activated in two or more subtraction pairs and excludes those areas that activate differentially in the pairs. E.g. in our first study, we looked for the common activation differences between retrieval 24 hours and 10 minutes after learning in two different verbal memory tasks. The revealed brain activations are functionally specific for the difference between time lags but general regarding the type of memory task.

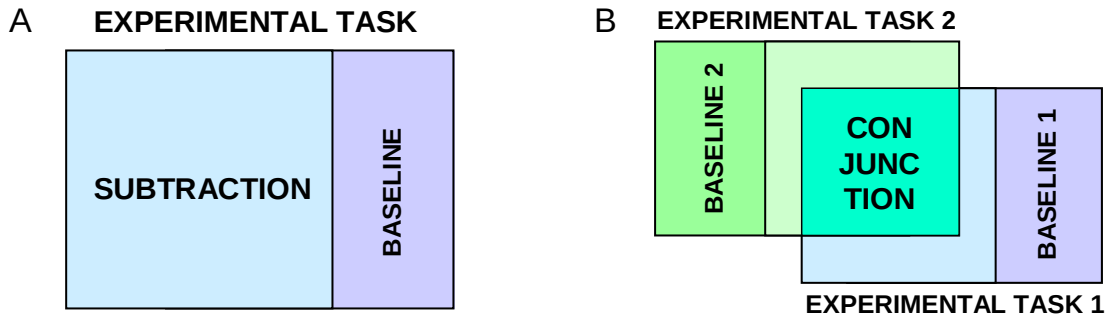

Fig. 6. Two different design types to measure cognitive function. In the cognitive subtraction approach (A) the process of interest is represented by the difference that results from the subtraction of the baseline activation from the activation elicited by the experimental task. In the cognitive conjunction approach (B), two or more subtraction pairs are combined and the process of interest is represented by the component that is common to all subtraction pairs (intersection).

1.2.3 The block design

The traditional approach of conducting an fMRI study is the so called block design (Fig. 5b). In a block design, brain activity is averaged over periods (epochs) of the experimental task that alternates with baseline periods. The optimal duration of an epoch is determined by the time course of the hemodynamic response and typically lasts from 16 to 25 s with the consequence that within one epoch usually several stimuli are presented. In many cognitive tasks, not every stimulus in a block elicits the same cognitive processes in a participant. In memory tasks for example, one can distinguish between correctly recalled and forgotten items or between Remembered and Known items. In a blocked design activity is averaged over different cognitive processes that can occur during a specific task (Maguire and Frith, 2003). A major advance was the development of event related fMRI which allows to sort and average the brain activity of events based on participants responses such as whether a stimulus was recognized or not (for a review of event-related fMRI see Rosen et al., 1998). However, in an event-related design a high number of stimuli (at least 30) must be presented, a requirement that cannot be met by all types of cognitive tasks. In paired associative learning and retrieval tasks for example, the number of stimuli that can be learned and retrieved lies far below the required minimum of an event-related design. Moreover, the signal detection power of event-related fMRI is much lower than that in a block design (Liu et al., 2001). Unfortunately, in the medial temporal lobe the magnitude of the signal is much lower than in primary sensory areas (less than 1% vs. 2-3%) (Cohen and Bookheimer, 1994), making it a non-optimal target for event-related fMRI. Thus, event-related fMRI is a valuable tool for the measurement of perceptual functions in which a high number of stimuli can be presented and the loss of signal detection power can be afforded, in contrast to associative memory tasks in which these limitations can lead to serious disadvantages.

2. 2. THE PRESENT EXPERIMENTAL STUDIES

2.1 General questions and hypotheses

The two studies were designed to compare retrieval-associated cerebral activations of recent versus remote memories with time lags of 10 minutes, 1 day and 1 month. These time lags are shorter than the typical long-term delays used in the existing systems consolidation literature. The present theories on human systems consolidation make no predictions for consolidation-related changes in such short time periods and the existing data regarding this time range are very sparse (Stark and Squire, 2000). However, animal studies on systems and cellular consolidation demonstrated that effects of memory consolidation occur after time lags of several hours up to weeks (see 1.1.2.1 and 1.1.5). Therefore, it seems appropriate to investigate the change of retrieval-related brain activations over such short intervals by means of fMRI in humans.

2.1.1 Main questions:

- **Can we measure effects of memory consolidation after study-test intervals of 1 day and 1 month with functional magnetic resonance imaging?**
- **How do our findings contribute to the discussion about the role of the MTL in the retrieval of consolidated memories?**

2.1.2 Hypotheses of the first study:

In the first study, we wanted to test whether short term consolidation over 24 hours had an impact on retrieval related signal changes in fMRI. The time lags used in this study based on the findings in animals where structural synaptic changes due to consolidation processes have been found 24 hours after learning (O'Malley et al., 2000; Leuner et al., 2003). Recent literature on the neurophysiological basis of the fMRI signal (Magistretti and Pellerin, 1999; Logothetis et al., 2001; Bonvento et al., 2002; Lauritzen and Gold, 2003) demonstrated that the BOLD signal measures synaptic activity.

- **We will detect a change in fMRI signal when comparing memory-retrieval-related activities 24 hours versus 10 minutes after learning.**
- **The change will be an increase in signal in the MTL at the 24-hour- versus the 10-minute-retrieval. This increase may reflect the neurobiological findings of an increased potential for synaptic connectivity in the first 24 hours after learning.**

2.1.3 Hypotheses of the second study:

In the second study, we increased the delay time to 1 month and compared brain activations during retrieval after 1 month versus 1 day. Basis for this time interval was the study of Bontempi et al. (1999) who found a decrease of memory retrieval related metabolic activity in the MTL of rats 25 days after learning. Further, we were interested in whether fMRI activity at the 1-day-lag would predict later performance and the ability to retain information over 1 month. We assume that stronger activity at the 1-day-lag reflects a better integration of information in the memory network and therefore enables the retention of the information over longer time.

- **We will find a decrease in retrieval-related hippocampal activity from the 1-day- to the 1-month-retrieval as a result of the time-limited role of the hippocampus in memory retrieval.**
- **MTL activation during the 1-day-retrieval predicts the amount of retention over 1 month. The stronger the activity at the 1-day-lag, the more information will be retained over 1 month.**

2.2 The two studies in short

2.2.1 Common design features

On the core of both studies was the prospective design which allowed controlling the variables that are confounded in so many of the traditional consolidation studies: encoding, rehearsal, and quality of retrieval. To this aim we used the following approaches: First, we employed an incidental encoding paradigm to ensure that all participants used the same encoding strategy. Second, we let participants fill out a questionnaire about whether they rehearsed the learned material in the time between encoding and retrieval to have some control over the intervening time. Third, quality of retrieval was measured with the Remember/Know paradigm (Tulving, 1985). Fourth, we empirically and statistically equalized the amount and quality of retrieval between the two time lags (for detailed information about these strategies see control of confounding variables 2.3).

The multiple trace theory states that only information that was dependent on hippocampal function in the first place remains reliant on this structure during the course of consolidation (Nadel and Moscovitch, 1997). This precondition makes it crucial to have a task that is known to rely on the hippocampus. Importantly, the hippocampus is most challenged by associating primarily unrelated items (Henke et al. 1997, 1999). Therefore, we used the learning of word-word associations.

In both studies, learning took place on two different sessions that preceded the single retrieval session with the required time interval (Fig. 7a). The learning stimuli were always a pair of visually presented unrelated abstract nouns (Fig. 7b).

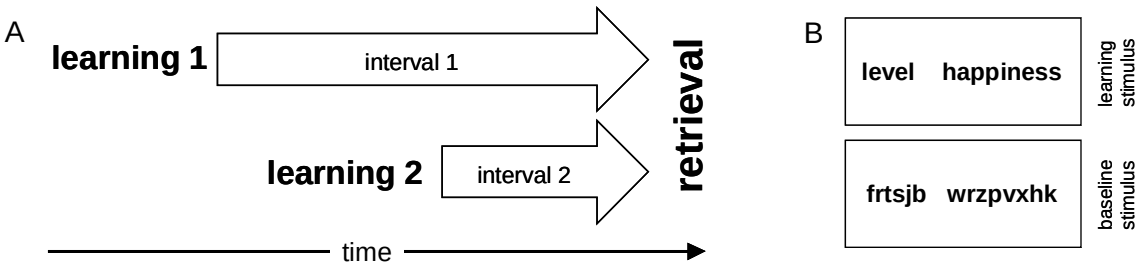

Fig. 7. General design (A) and Stimulus examples (B). A: Learning is divided into two sessions such that it precedes retrieval with either interval 1 or interval 2. B: Experimental and baseline stimuli were presented in the same font and size.

The word pairs were taken from the study of Henke et al. (1999) who selected them on grounds of a behavioural pilot study which demonstrated that each two words of a pair were a priori not associated. In both our studies, learning tasks were presented as language tasks so that participants did not expect a later test for retrieval. Retrieval stimuli were either exactly the same word pairs as during learning (Old word pairs) or New word pairs made out of a recombination of old words. Retrieval was tested with a recognition task which consisted of 75% Old and 25% New word pairs. During retrieval, concurrent fMRI measurement took place. The baseline stimuli included for the fMRI block design (1.2.3) consisted of two unpronounceable consonant strings, presented in the same font and size as the word pairs (Fig. 7b). Yet, they did not contain meaning to avoid semantic encoding and thus hippocampal activity.

In both studies, only healthy participants were included. All of them gave informed consent after the nature and possible consequences of the behavioural experiment and fRMI measurement was explained.

2.2.2 First study

In this study, we compared the fMRI signal during the retrieval of word pairs learned 24 hours versus 10 minutes previously.

We used high- and low-associative learning and retrieval of word pairs (Henke *et al*., 1999) and were primarily interested in time-related commonalities between the two types of learning. High- and low-associative learning and retrieval primarily differed by their learning instructions. High-associative learning induced the formation, storage and retrieval of many semantic word-word associations whereas low-associative learning emphasized on the processing of the two words individually and induced the formation, storage and retrieval of only a few associations between the two words. We used these two different types of verbal learning with two different learning instructions to ensure that activation differences are task unspecific and not related to the type of learning instruction. The commonalities were analyzed by use of a conjunction analysis (1.2.2).

The results revealed a main effect of time of an enhanced left hippocampal activity during the retrieval after 24 hours versus 10 minutes. This increase was also expressed in an enhanced relationship between retrieval performance and the fMRI signal in the MTL during the retrieval of word pairs learned with the high-associative and the low-associative instruction. The results are in agreement with findings in animals that suggest a strengthening of synapses in the hippocampus within 24 hours after learning.

2.2.3 Second study

In the second study, we compared brain activations during retrieval after 1 month versus 1 day.

We focused on high associative learning and retrieval in a group of 12 good learners and 8 poor learners. A high-effective and a low-effective learning procedure were administered during both learning sessions preceding the retrieval. These two learning procedures had the following rationale: The 1-month-retrieval after the high-effective learning procedure resulted in equal retrieval quality as the 1-day-retrieval after the low-effective learning procedure. Thus, we could compare brain activity

during retrieval after 1 day versus 1 month with equal learning procedures and moreover with equal retrieval quality.

Good learners showed for the day- and the 1-month-lag an equally high retrieval performance. Poor learners did not differ from the good learners at the 1-day-lag, but showed a marked decrease in retrieval performance over 1 month, i.e. the high-effective learning procedure did not help them retain the learning material over one month.

In good learners, we found for both the comparison with equalized learning procedures and the comparison with equalized retrieval quality an increase in retrieval-related fMRI signal in the MTL after 1 month versus 1 day, suggesting that there is no decrease of hippocampal involvement in memory retrieval over 1 month. In poor learners, the picture was reversed with an increase after 1 day versus 1 month. Importantly, the poor learners dramatic performance decrease explains the decrease in MTL activity over 1 month. Further, the strength of the fMRI signal in bilateral hippocampi at the 1-day-lag predicted the amount of forgotten word pairs after 1 month, i.e. the stronger the signal at the one-day-retrieval, the higher was the decrease in performance from the 1-day- to the 1-month-retrieval.

The increase in signal in the MTL in the group that was able to retain the learned word pairs over the long interval of 1 month might suggest that the hippocampus further intensifies its engagement in the retrieval of consolidated information. Conversely, there is a clear decrease in hippocampal involvement when information is forgotten, as observed in the group of poor learners.

Second, the fMRI signal at the 1-day-lag predicted the outcome of memory retrieval after one month. However, a strong signal at the 1-day-retrieval was not related to good retention capacity but to the amount of forgotten word pairs from the 1-day- to the 1-month-retrieval.

2.3 Control of confounding variables

2.3.1 Incidental learning

How the presented stimuli are learned is very important with regard to what is later retrieved and how well it is retrieved. When learning is intentional, i.e. participants are told that they later have to retrieve the word pairs, each participant uses a different encoding strategy. Some of them would encode the word pairs very deeply with many semantic associations, others rather shallowly relying on visual/perceptual or phonological characteristics of the presented words only. Incidental learning on the other hand means that participants are not aware of later retrieval. The task instructions are not to learn something but to process the presented material in a given and therefore for all participants equal way. In verbal learning paradigms, tasks can be disguised as language tasks, e.g. to find an association between the two words of a pair or to create a meaningful sentence containing both words. These instructions foster a deep semantic processing of the two words. As soon as new information is processed it is also automatically encoded by the hippocampal complex. Thus, the hippocampus is not only active when participants know that they have to learn but whenever new material is presented. This explains also why we remember things without having them explicitly learned. E.g. I remember what I ate for breakfast although I had not intended to retrieve it later.
In addition, incidental encoding does not encourage participants to rehearse the material in the time between learning and retrieval to improve their retrieval performance. However, it cannot be excluded completely that some of the participants would repeat the presented material for themselves or when telling someone else about the experiment. Therefore, upon debriefing we let participants fill out a questionnaire about whether they rehearsed or not. Fortunately, only two participants did so and hence could be excluded from statistical analyses.

2.3.2 Equalizing retrieval

As stated above (1.1.4), it is critical that the amount of retrieved information of recent and remote memories is the same. This prevents that the measured differences in fMRI signal can simply be explained by differences in the amount of retrieved items.

As memory traces naturally weaken over time, the only possibility to achieve an equal retrieval performance after different study-test intervals is to manipulate the strength of encoding in a way that the encoding procedure for the longer interval results in stronger traces than that for the shorter interval. This way, the greater decay of the memory trace over the long interval may lead to the same net retrieval quality and quantity as the small decay over the short interval. To this aim, we conducted pilot studies for each of the two experimental studies to determine the exact learning instructions and numbers of learning runs that would result in an equal retrieval performance over the different delay intervals.

For the first experiment, we had to equalize two different types of learning between retrieval after 1 day and 10 minutes: high-associative learning and low-associative learning. The pilot study showed that for high-associative learning the manipulation of the depth of encoding by variation of learning instructions successfully equalized the retrieval of the two delay times. For the low-associative learning, an equal retrieval performance was achieved by means of increasing the number of learning runs during the learning session preceding retrieval 1 day.

Equalizing retrieval performance 1 day and 1 month following learning was more difficult because a considerable decay of the memory trace over the long interval of 1 month had to be expected. The pilot study to determine instructions and number of learning runs was therefore conducted as part of a diploma thesis (Billeter, 2003). The following important findings of this thesis had an impact on the design of the learning procedure of the second study: First, the low-associative learning instruction failed to produce a high enough retrieval performance for the 1 month delay. Therefore, in the second study, only high-associative learning was included. Second, a high number of learning runs did not necessarily improve retrieval because it was very repetitive and thus tiring. Instead, we found that separating the learning runs with interspersed semantic tasks was a better strategy, probably because it led to a short-term consolidation in the time between the runs and was in addition less tiring for the participants. Third, the number of word pairs per condition had to be reduced from 16 to 12 pairs to get an adequate retrieval performance.

2.3.3 Equalizing encoding

When retrieval performance is equalized by means of using different learning procedures for the long and the short intervals, changes in brain activation between the two retrieval delays could also be interpreted as a consequence of the different learning procedures instead of consolidation effects. Therefore, in the second study we administered a design which allowed comparing brain activations during retrieval 1 month versus 1 day following learning with either equalized retrieval quality or with equal encoding procedures.

2.3.4 Remember versus Know

Given that according to Tulving (1985) only remembering can strictly be regarded as episodic memory, it seems to be more important to equalize retrieval quality (only remembered memories) than retrieval quantity (memories based on recollection and feelings of familiarity only).

Therefore, we administered the Remember/Know procedure (Tulving, 1985) to have an idea whether the retrieved word pairs corresponded to episodic recollection or to retrieval made on a familiarity basis only (semantic memory).

The use of this procedure had a further advantage: Participants may perhaps discover the unequal distribution of 75% Old and 25% New word pairs during the retrieval task. As a result, they might give more frequently Know answers than New answers to maximize their performance. However, this possible bias of giving more Know answers has no impact on our episodic retrieval measure that includes Remember answers only.

3. ORIGINAL ARTICLES

A1 **Bosshardt, S.**, Schmidt, C. F., Jaermann, T., Degonda, N., Boesiger, P., Nitsch, R. M., Hock, C., and Henke, K. (in the press) Effects of memory consolidation on human hippocampal activity during retrieval.

A2 **Bosshardt, S.**, Degonda, N., Schmidt, C. F., Boesiger, P., Nitsch, R. M., Hock, C., and Henke, K. (manuscript in preparation) 1 month of memory consolidation enhances retrieval-related hippocampal activity.

Effects of memory consolidation on human hippocampal activity during retrieval

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Abbreviated Title: Effects of memory consolidation

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ABSTRACT

Day-to-day memories undergo transformation from short-term to long-term storage, a process called memory consolidation. Animal studies showed that memory consolidation requires protein synthesis and the growth of new hippocampal synapses within 24 h. To test for effects of memory consolidation in the human, we examined brain activation during the retrieval of information at 10 min and at 24 h following learning using functional magnetic resonance imaging (fMRI), an indirect measure of synaptic activity. Learning instructions were adjusted to yield a comparable retrieval quantity and retrieval quality at 10 min and 24 h after learning. The left hippocampal formation exhibited enhanced activity during the retrieval at the 24 h lag compared to the retrieval at the 10 min lag. Moreover, the activity in the left anterior hippocampal formation showed stronger correlations with retrieval quantity and retrieval quality at the 24 h lag than at the 10 min lag. This suggests that the relation between left anterior hippocampal activity and retrieval success became closer as consolidation progressed. These fMRI results in the human hippocampal formation may correspond to the neurobiological results in the animal hippocampal formation of a strengthening and growth of synaptic connections within 24 h.

INTRODUCTION

In cognitive neuroscience, the term memory consolidation refers to two-way interactions between medial temporal and neocortical regions for the long-term storage of explicit memories. One view of memory consolidation posits a time-limited role of the hippocampus for the retrieval of declarative information with memories eventually becoming entirely supported by neocortical structures (Squire and Zola-Morgan, 1991; Squire and Alvarez, 1995). The multiple trace theory (MTT), on the other hand, suggests that the hippocampus is continuously involved in the retrieval of episodic information and that over time and upon reactivation multiple related memory traces are formed in the hippocampus (Nadel and Moscovitch, 1997; Nadel et al., 2000). These so called systems consolidation processes are time-consuming and in the human may take months or years to completion (e.g. Knowlton and Fanselow, 1998).

In neurobiology, memory consolidation refers to molecular and cellular events that fix preliminary alterations in synaptic strength in order to transform fragile memory traces into more stable representations. These processes occur within hours after learning. Long-term retention requires that within 24 h after learning gene expression be activated to give rise to the synthesis of new proteins and possibly to the growth of new synaptic connections in the hippocampal formation and other memory systems (Milner et al., 1998; Kandel, 2001).

While the molecular and cellular underpinnings of memory consolidation within the first 24 h after learning have been vigorously investigated in animals, research on the 24 h memory consolidation in the human is sparse. Yet, it is possible to indirectly measure retrieval-related brain activity in the human with the blood-oxygen-level-dependent (BOLD) fMRI (functional magnetic resonance imaging) method. The BOLD response is believed to relate to changes in excitatory (glutamatergic) neurotransmission and can be considered an indirect measure for all aspects of presynaptic and postsynaptic processing (Magistretti and Pellerin, 1999; Logothetis et al., 2001; Raichle, 2001; Bonvento et al., 2002; Lauritzen and Gold, 2003; Logothetis, 2003; Zonta et al., 2003).

The two standard approaches in neuroimaging studies of human memory consolidation and retrieval were to either have participants learn items and retrieve them only minutes after learning in the same session (Lepage et al., 1998; Schacter

and Wagner, 1999) or to have participants retrieve autobiographical information from the past (e.g., Haist, 2001; Maguire et al., 2001). The problem with retrospective studies of memory consolidation is that it remains unknown exactly when information was acquired, how well it was learned, or how often and when the last time a certain memory was retrieved and thus re-encoded. Moreover, memories may change character over time. Autobiographical memories may become less episodic ('event'-like, rich in details, authentic, personal) and more semantic ('fact'-like) over time (e.g., Cermak and O'Connor, 1983). The final representational status of the tested memories should be equivalent across time lags if brain activity differences at test are to reflect effects of time lag rather than effects of memory systems. Prospective studies allow investigators to determine with much greater precision the time, strength, and nature of learning, and - to some degree - the rehearsal of the learning material between study and test.

With such a prospective approach and a comparable retrieval performance between time lags, Stark and Squire (2000) found a non-significant trend towards a retrieval-related fMRI signal increase in the left and right anterior and posterior hippocampal region at 24 h versus 30 min following study. They found no such activity increase if retrieval performance remained unequated between study-test intervals. The same negative result was obtained by Dupont et al. (2001) who had participants silently recall the same list of words 20 min and 24 h following learning without controlling for performance differences in the retrieval across time lags.

In this study, we indirectly measured retrieval-related brain activity with BOLD fMRI 10 min and 24 h after the experimentally controlled learning of word pairs. Our analyses focused on time lag-related activity differences within the medial temporal lobes (MTL). The following methodological considerations were applied:

First, our interest concerned common effects of time lag across several hippocampal memory tasks avoiding task-specific effects. We therefore looked at the main effect of time lag in a two (time lag) - by - two (memory task) ANCOVA and at the cognitive conjunction of memory tasks (Price and Friston, 1997; Cabeza et al., 2002). We used two paired-associate word learning tasks, a high-associative and a low-associative task, that differed in their efficacy of establishing associations in long-term memory. Both task instructions fostered a deep encoding of the single words. However, while the low-associative task instruction provided for a poor learning of additional semantic word-word associations, the high-associative task instructions

automatically led to the establishment of many semantic word-word associations (Henke et al., 1999).

Second, we used incidental learning instructions to avoid intentional rehearsing and re-encoding of the word pairs between study and test.

Third, different lists of word pairs were used for each task and each study-test interval to avoid repetition effects and re-encoding due to repeated testing.

Fourth, we empirically and statistically controlled for a comparable retrieval quality and quantity between the two time lags. This is important because the natural process of forgetting might lead to weaker memory traces and thus a bias in the retrieval-related fMRI response at the 24 h lag compared to the 10 min lag. The empirical control of retrieval performance can be achieved by increasing the number of learning trials (e.g., Stark and Squire, 2000) or by varying the learning instructions to adjust the depth of stimulus processing (Heckers et al., 2002). We used the first method for the low-associative memory task and the second method for the high-associative memory task. The ANCOVA and the conjunction analysis allowed testing for common effects of time lag on retrieval, independently of the specific learning procedures used.

Fifth, even with the number of retrieval events equated, the quality of the retrieved information (‘event’-like, ‘fact’-like) may still largely differ between time lags. We therefore assessed and statistically controlled the quality of the retrieved information by using Tulving’s (1985) Remember/Know procedure. Accordingly, Old (studied) - New (not studied) decisions in the test given for recognition were followed by Remember/Know decisions for Old answers. A Remember response implied that the whole study episode was recollected (e.g., one’s own thoughts at the first encounter with an item). A Know response implied item familiarity without recollection of the study episode. It has been suggested that the hippocampal formation, defined here as the CA regions, dentate gyrus and subicular complex (Cohen and Eichenbaum, 1993), mediates recollection while the perirhinal cortex mediates familiarity-based recognition (Eldridge et al., 2000; Brown and Aggleton, 2001; Yonelinas et al., 2002; Davachi et al., 2003). On the other hand, Remember and Know answers have been considered qualitatively equal, hippocampal-dependent recognition processes which simply differ in the amount of the retrieved information (Manns et al., 2003). Whichever account is favored, the control for potential

Remember/Know differences between time lags appears essential for the study of time lag effects on retrieval-related hippocampal activity.

These precautions allowed us to isolate relatively pure effects of study-test intervals on retrieval-related hippocampal activity.

MATERIALS AND METHODS

Participants

Twelve healthy right-handed volunteers (seven men) of different educational levels (9-18 school years; mean: 14.8, SD: 2.7) and ages (20-45 years; mean: 27.6, SD: 6.9) participated in this study. Participants were either students at the University of Zurich or worked at the financial services department of a Swiss bank where advertisements of this study were posted. Participants had above-average intelligence and good learning capacities as assessed in an extensive mental status examination which included the Wechsler Memory Scale Revised (general memory score: M: 120.8, SD: 19.2; delayed retrieval score: M: 124.6, SD: 13.6) and the Wechsler Adult Intelligence Scale Revised (mean Full Scale IQ 140.3; SD: 7.9; mean Verbal IQ: 136.5, SD: 9.2; mean Performance IQ: 132.3, SD: 9.5). Informed written consent was obtained after the nature and possible consequences of the fMRI study were explained. The study protocol was approved by the local ethics committee for human studies.

Stimuli

For each learning/retrieval condition, we used a separate stimulus set consisting of 16 pairs of unrelated abstract nouns (Fig. 1B) taken from a previous study (Henke et al., 1999). The stimulus sets were counterbalanced across experimental conditions. In the baseline condition, the stimulus set contained 16 pairs of unpronounceable consonant strings (Fig. 1B) which had an equal average length as the abstract nouns given for study in the experimental conditions. The sequence of consonants per string was randomly generated.

Memory Tasks

The experiment consisted of two learning sessions with each separate learning material and a retrieval fMRI session. The first learning session took place on day

one. The second learning session was conducted exactly 24 h later on day two and was 10 min later followed by the retrieval fMRI session. For the retrieval fMRI session, both the learning material of day one and day two were presented again for recognition. This way, half the learning material was retrieved 10 min after learning and half 24 h after learning.

Learning tasks

All learning tasks were practiced ahead of the experiment until participants were at ease with the tasks. Learning sessions on both days included two incidental learning tasks: a high-associative and a low-associative task with each separate learning material.

The learning instruction for the *high-associative task* given on day one was to generate a meaningful sentence out loud which contained both nouns of a pair. There were no time limits for sentence generation. This task was given twice (two runs) with the same list of words. The learning instruction for the high-associative task given on day two was to silently find a semantic relation between the two nouns of a pair and then indicate by button press whether the semantic relation found was rather close (left button) or far (right button). Participants were given six sec per word pair. This task was also given twice (two runs) with the same list of words. Both the learning instruction given for day one and the learning instruction given for day two fostered the formation and establishment of semantic associations between the primarily unrelated nouns of a pair. Pilot studies had shown that the sentence generation task yielded a comparable associative retrieval performance after 24 h as the semantic relatedness task after 10 min.

We used the same learning instruction for the *low-associative task* given on day one as for the low-associative task given on day two. Yet, we varied the number of learning runs between days to achieve a comparable retrieval performance between time lags. This common learning instruction required participants to decide, and then answer by button press, whether each single noun of a pair was rather pleasant or unpleasant. Participants pressed the right key if one of the two nouns was pleasant and the left if both or none was pleasant. Word pairs were presented for six sec during which time participants were to answer by button press. They engaged in four learning runs with the same list of words on day one and in two learning runs with the other list of words on day two. The learning instruction used for the low-associative task (pleasant/unpleasant) had already been used in the study of

Henke et al. (1999) who found that this instruction yielded the establishment of only few semantic word-word associations in memory but yielded a deep encoding and a good retrieval of the individual nouns. Thus, although well suited for the memorization of the individual nouns, this learning instruction yields a poor learning of semantic associations. In the current study, we tested only for the retention of semantic word-word associations, not individual words. Importantly, all learning tasks were incidental and passed as language-related tasks to keep participants from rehearsing and re-encoding the learning material between study and test.

Learning procedure

All participants were examined at a time between 1:00 p.m. and 7:00 p.m. on day one and two. Participants were scanned on day two only. On day one, participants learned the first two lists of word pairs - one list with the high-associative task (sentence generation) and the other list with the low-associative task (pleasant/unpleasant decision). Half the participants started with the high-associative task and half with the low-associative task. The runs of one task were interleaved with the runs of the other task to create some spacing between the runs belonging to the same task and word list (see Fig. 1A).

The same procedure was used for day two (Fig. 1A). For the day 2 learning session, participants were already situated in the MR scanner to keep the time interval of 10 min between study and test. Participants learned two further lists of word pairs - one list with the high-associative task (find semantic relation) and the other list with the low-associative task (pleasant/unpleasant decision). Half the participants started with the high-associative task and half with the low-associative task. Runs per task were again interleaved. Participants were free to form new sentences and to change their semantic relatedness and pleasant/unpleasant decisions from run to run.

Retrieval task

The retrieval of all learning material was assessed during a single fMRI time-series with blocked trials by use of the same recognition procedure for all four word lists (Fig. 1A, B).

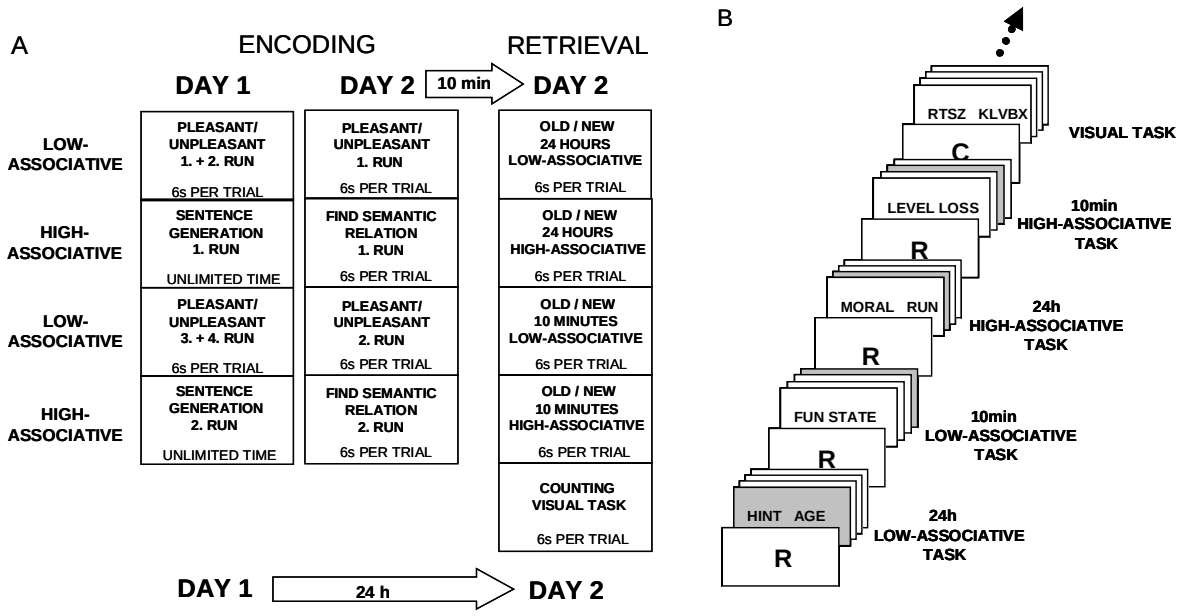

Figure 1. Task procedure (A) and fMRI design (B). A: There was a learning session on day one and a second learning session (with different learning material) on day two. Retrieval of all learning material was carried out on day two, i.e., 24 h after the first and 10 min after the second learning session. Each learning session included a high-associative and a low-associative incidental learning task with either two or four learning runs on 16 word pairs. The runs per learning task were presented in an interleaved manner. Learning instructions were 1) to judge the pleasantness of individual words (Pleasant/Unpleasant), 2) to generate a sentence which contains both words of a pair (Sentence Generation) and 3) to find a semantic relation between the words of a pair (Find Semantic Relation). The retrieval instruction was to decide whether a word pair was Old or New (Old/New). Old answers were followed by a Remember/Know decision. A visual task was included as a baseline condition in the retrieval fMRI time-series with the instruction to count (Counting) the angular-shaped consonants across pairs of consonant strings. B: The retrieval fMRI time-series included blocked trials of the four retrieval conditions and the visual baseline condition. There were four epochs per condition (the illustration shows only one task cycle). One out of the four trials per retrieval epoch contained a New word pair (grey shaded for illustration). The first slide of each epoch was an instruction slide (R, Retrieval; C, Counting).

Two word lists had been learned with the high-associative task and two with the low-associative task. For recognition, we presented 75% Old (presented for learning) word pairs and 25% New word pairs (new combinations of old words) per epoch and task. Participants were required to answer by button press whether a word pair was Old (left button) or New (right button). Old decisions were followed by a second button press which indicated whether a word pair was actually Remembered (left button) or just Known (right button) (Tulving, 1985). The choice of Remember implied that the specific learning episode for a given word pair was re-experienced and the choice of Know implied that the word pair only evoked a feeling of familiarity. The Old/New task assessed retrieval *quantity* and the Remember/Know task retrieval

quality. This fMRI time-series also included a baseline condition consisting of a task that required a low-level visual analysis of pairs of unpronounceable consonant strings (Fig. 1B). The instruction was to count angular-shaped consonants across the strings of a pair and indicate their number by button press (left button: ≤ 4; right button> 4). We used consonant strings rather than real words because these neither contain meaning nor bear a phonological resemblance to meaningful words. This was a precaution to avoid the semantic encoding of new word pairs during the baseline condition.

fMRI Design

fMRI data were collected during retrieval in a single time-series over eight min and 40 sec with blocked trials per condition (see Fig. 1B). There were 20 epochs - four epochs for each of the five conditions. Each epoch lasted 26 sec and comprised the presentation of four stimuli (six sec per stimulus) and an instruction slide displayed for two sec at the beginning of each epoch. Epochs were alternated in a fixed order. The sequence of conditions was varied across participants. A retrieval epoch consisted of three Old and one New word pair. The sequential position of the New among the Old word pairs of an epoch was systematically varied across epochs. The computer-driven stimulation was projected with an LCD-projector to the back of a screen that participants could watch through mirrors attached to the head coil.

Image Acquisition and Post-processing

Functional T2*-weighted images were acquired on a 3 Tesla MR Imaging system (Philips Medical Systems Inc, Best, The Netherlands) with an echo-planar pulse sequence (EPI) from 32 axial slices. The slices were scanned continuously with an acquisition matrix of 80 x 80 (voxel size 2.8 x 2.8 x 4 mm^3) and were reconstructed to an image matrix size of 128 x 128 (voxel size 1.7 x 1.7 x 4 mm^3). Acquisition parameters were TR 4500 ms, flip angle 90°, TE 30 ms, no slice gap.

Structural reference images were obtained from a T1-weighted 3D scan with a matrix size of 224 x 224 (voxel size 1.0 x 1.0 x 0.75 mm^3) which was reconstructed to an image matrix size of 256 x 256 (voxel size 0.9 x 0.9 x 0.75 mm^3; TR 20 ms, flip angle 20°, TE 2.3 ms, inter-slice gap 0.75 mm).

Image post-processing and the statistical analyses were performed using Statistical Parametric Mapping (SPM99; http://www.fil.ion.ucl.ac.uk/spm/). Volumes were realigned to the first volume to remove movement-related variance components. Images from all participants were spatially normalized into standard stereotaxic space (standard EPI template SPM99) (Friston et al., 1995). Finally, data were smoothed to a full width of 8 mm at half-maximal resolution using a Gaussian filter.

Statistical Analysis of fMRI Data

Single-subject level

The fMRI data of the 12 participants were analyzed voxel by voxel modeling the conditions as box car functions convolved with a hemodynamic response function and applying the general linear model (fixed-effects analysis; Cohen, 1997) provided in SPM99.

Group level

We performed three types of group analyses: analyses of covariance (ANCOVA), conjunction analyses, and correlation analyses. Height thresholds were $p = 0.001$ (uncorrected for multiple comparisons) and extent thresholds were four voxels.

Two - by - two ANCOVA: To assess activity differences between the 24 h and the 10 min retrieval over both memory tasks, we computed the main effect of time lag in a two (time lag) - by - two (memory task) ANOVA for each participant. The two resultant within-subject contrasts (two one-tailed tests) for the main effect of time lag were then entered into two ANCOVA and tested against zero at the second level. The covariate for the main effect of time lag was the difference in the number of correctly Remembered (Tulving, 1985) word pairs between the 24 h lag and the 10 min lag added over both memory tasks. This covariate was included to statistically control for differences in retrieval quality between time lags in the face of an insufficient empirical control of retrieval quality in the high-associative task. The empirical control of both retrieval quantity and retrieval quality was good in the low-associative task.

One-way ANCOVA: For the separate comparison of high-associative retrieval and low-associative retrieval between time lags, we computed fixed-effects analyses for each participant comparing the 24 h retrieval with the 10 min retrieval (and vice versa) for each of the two memory tasks. The four resultant within-subject contrasts were then entered into four ANCOVA and tested against zero at the second level with the covariate "difference in the number of correctly Remembered (Tulving, 1985) word pairs between the 24 h lag and the 10 min lag".

Conjunction analyses: To test for larger activities at the 24 h than the 10 min retrieval (and vice versa) which are *shared* by both memory tasks, we computed two conjunction analyses (Price and Friston, 1997; Cabeza et al., 2002), one for each direction of activity differences. To this aim, we entered the within-subject contrasts between the 24 h retrieval and the 10 min retrieval (and vice versa) for each task into a one-way (time lag) ANCOVA and tested differences against zero at the second level. The covariate for each task was the difference in the number of correctly Remembered (Tulving, 1985) word pairs between the 24 h lag and the 10 min lag in the respective task. A conjunction of the resultant activation maps (thresholded at p = 0.033) was computed by multiplying the two activation maps with the ImCalc feature of SPM. The joint probability threshold p was 0.033 x 0.033 = 0.001 (e.g., Cabeza et al., 2002).

Correlation of the fMRI signal with retrieval performance*:* To assess the strength of the relationship between brain activity and retrieval quantity or retrieval quality for each task and each time lag, we correlated the within-subject contrasts (retrieval versus baseline condition) resulting from the fixed-effects analyses (computed for each participant) with either retrieval quantity (hits plus correct rejections in %) or retrieval quality (# correctly Remembered word pairs).

RESULTS

Behavioral Data

Effects of task

Retrieval quantity (Fig. 2A) (hits plus correct rejections in %) was significantly better in the high-associative (HA) than the low-associative (LA) task at both the 24 h lag (HA: M = 77 %, SD = 7 %; LA: M = 63 %, SD = 15 %; t (11) = -4.1, p (2-tailed) =

0.002) and the 10 min lag (HA: M = 75 %, SD = 7 %; LA: M = 63 %, SD = 11 %; t (11) = -2.7, p = 0.022).

Retrieval quality (Fig. 2B) (# correctly Remembered word pairs) (Tulving, 1985) was significantly better in the high-associative than the low-associative task at both the 24 h lag (HA: M = 8.3, SD = 1.7; LA: M = 4.3, SD = 2.6; t (11) = -4.7, p (2-tailed) = 0.001) and the 10 min lag (HA: M = 6.8, SD = 2.0; LA: M = 4.4, SD = 3.0; t (11) = -2.3, p = 0.03).

Effects of time lag

Retrieval quantity (Fig. 2A) (hits plus correct rejections in %) was comparable between time lags for both the high-associative task (t (11) = 0.44, p (2-tailed) = 0.67) and the low-associative task (t (11) = 0.12, p = 0.91). The percentage of false alarms was also comparable between time lags for each task (high-associative task: M_{24h} = 33.3, SD_{24h} = 26.8; M_{10min} = 31.3, SD_{10min} = 18.8; t (11) = 0.32, p (2-tailed) = 0.75; low-associative task: M_{24h} = 43.8, SD_{24h} = 24.1; M_{10min} = 41.7, SD_{10min} = 26.8; t (11) = 0.23, p = 0.82).

Retrieval quality (Fig. 2B) (# correctly Remembered word pairs) (Tulving, 1985) was comparable between time lags for the low-associative task (t (11) = -0.09, p (2-tailed) = 0.93) but was almost significantly better at the 24 h lag than the 10 min lag for the high-associative-task (t (11) = 2.14, p = 0.056).

The total number of given Old responses (hits plus false alarms) and thus the number of Remember/Know judgments was comparable between time lags for the high-associative task (M_{24h} = 11.2, SD_{24h} = 2.2; M_{10min} = 10.7, SD_{10min} = 2.1; t (11) = 0.58, p (2-tailed) = 0.57) and for the low-associative task (M_{24h} = 9.7, SD_{24h} = 2.3; M_{10min} = 9.5, SD_{10min} = 2.4; t (11) = 0.21, p = 0.84).

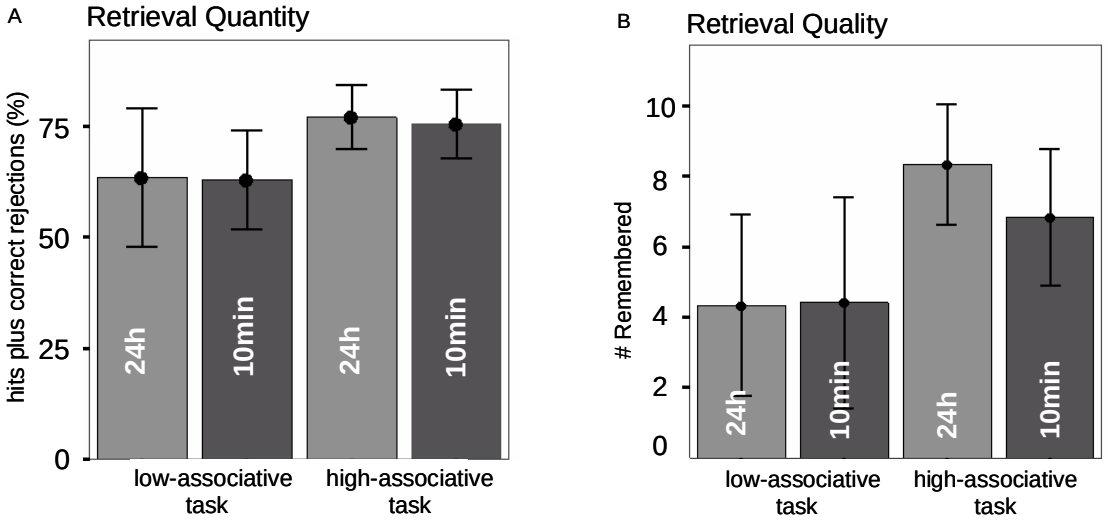

Figure 2. Retrieval quantity (A) and retrieval quality (B). A: The performance measure for retrieval quantity was hits plus correct rejections in % (possible range: 0 % - 100%). B: The performance measure for retrieval quality was the number of correctly Remembered (Tulving, 1985) word pairs (possible range: 0 – 12). Bars represent standard deviations.

Reaction times: Neither did reaction times for correct answers (hits plus correct rejections) nor false answers (misses plus false alarms) significantly differ between the 24 h retrieval and the 10 min retrieval in the low-associative task (correct answers: M_{24h} = 3076 ms, SD_{24h} = 828 ms; M_{10min} = 2962 ms, SD_{10min} = 654 ms; t (11) = 1.06, p (2-tailed) = 0.31; false answers: M_{24h} = 3511 ms, SD_{24h} = 511 ms; M_{10min} = 3432 ms, SD_{10min} = 757 ms; t (11) = 0.53, p = 0.61). Neither did reaction times for false answers significantly differ between the 24 h retrieval and the 10 min retrieval in the high-associative task (false answers: M_{24h} = 3579 ms, SD_{24h} = 531 ms; M_{10min} = 3487 ms, SD_{10min} = 790 ms; t (11) = 0.43, p = 0.68). However, reaction times for correct answers were significantly shorter during the 24 h retrieval than the 10 min retrieval in the high-associative task (M_{24h} = 2784 ms, SD_{24h} = 474 ms; M_{10min} = 2994 ms, SD_{10min} = 536 ms; t (11) = -2.32, p = 0.040).

Reaction times were comparable between time lags for the low- and the high-associative task if false and correct answers were combined (low-associative task: M_{24h} = 3248 ms, SD_{24h} = 693 ms; M_{10min} = 3137 ms, SD_{10min} = 652 ms; t(11) = 1.59, p = 0.14; high-associative task: M_{24h} = 2948 ms, SD_{24h} = 472 ms; M_{10min} = 3101 ms, SD_{10min} = 566 ms; t(11) = -1.58, p = 0.14).

Rehearsal between study and test: Upon debriefing, none of the participants reported to have expected a memory test or to have intentionally rehearsed the learning material between study and test.

fMRI Data

2 (time lag) - by - 2 (memory task) ANCOVA

Main effect of time lag: The comparison of the 24 h retrieval with the 10 min retrieval over both memory tasks revealed significantly enhanced activity in the left hippocampal formation (Table 1, Fig. 3A). As illustrated in Fig. 4A, this effect resulted from stronger activations in all four retrieval conditions relative to the baseline condition, with the two 24 h retrieval conditions yielding even stronger activity than the two 10 min retrieval conditions. Further activity differences between the 24 h retrieval and the 10 min retrieval were situated in the right superior frontal gyrus (BA 10), the left inferior temporal gyrus (BA 37) and the right fusiform gyrus (BA 19) (Table 2).

Table 1. Enhanced activity in the MTL during retrieval at 10 min vs. 24 h after learning and at 24 h vs. 10 min after learning as well as correlations between MTL activity and retrieval success at each time lag (height threshold p= 0.001; extent threshold k= 4 voxels).

Region of activation	Statistics	x	y	z	# vx	T	x	y	z	# vx	T
	comparisons of means	10 min vs. 24 h					24 h vs. 10 min				
L. Hippocampus	main effect						-24	-26	-12	14	4.6
R. Hippocampus	main effect	28	-36	-4	60	4.8					
	conjunction	29	-38	-7	27	2.7					
	correlations	10 min					24 h				
L. Hippocampus	HA – quantity						-28	-12	-18	8	5.6
R. Hippocampus	HA – quantity						26	-22	-18	47	7.0
L. Hippocampus	HA – quality						-24	-12	-16	5	5.6
L. Entorhinal c.	HA – quality						-28	-30	-24	4	7.0
L. Perirhinal c.	LA – quantity						-42	-22	-26	6	5.5

c, cortex; L, left; R, right; (x,y,z), MNI coordinates; vx, voxels; HA, high-associative task; LA, low-associative task

Table 2. Activity increases in the neocortex during retrieval at 10 min vs. 24 h after learning and at 24 h vs. 10 min after learning (height threshold p= 0.001; extent threshold k= 4 voxels).

Region of activation	Statistics	x	y	z	BA	T	x	y	z	BA	T
		10 min vs. 24 h					24 h vs. 10 min				
R. fusiform g.	main effect	28	-82	-18	19	5.4	36	-68	-12	19	5.3
	conjunction	28	-84	-19	19	2	36	-70	-15	19	3.6
L. inferior temporal g.	main effect						-42	-66	-2	37	6.6
R. superior frontal g.	main effect						8	60	34	10	4.7
L. orbital g.	main effect	-28	38	-8	11	5.1					
L. middle frontal g.	main effect	-34	24	30	9	4.4					
	conjunction	-37	24	26	9	5.3					
L. inferior frontal g.	conjunction	-37	36	15	45	4.0					
		-32	40	-9	47	4.0					
R. insula	main effect	32	-16	20		9.4					
L. precuneus	main effect	-8	-62	26	7	4.7					
L. inferior parietal l.	main effect	-34	-54	46	40	4.6					
R. inferior parietal l.	conjunction	47	-46	49	40	2					

g, gyrus; l, lobule; L, left; R, right; (x,y,z), MNI coordinates; BA, Brodmann Area

The reversed comparison (10 min retrieval versus 24 h retrieval) over both memory tasks yielded significantly enhanced activity in the right posterior hippocampal formation (Table 1, Fig. 3B). As illustrated in Fig. 4B, this effect resulted from weaker activations in all four retrieval conditions relative to the baseline condition, with the two 24 h retrieval conditions yielding even weaker activity than the two 10 min retrieval conditions. Further activity differences between the 10 min retrieval and the 24 h retrieval were situated in the right insula, the left middle frontal gyrus (BA 9) and left orbital gyrus (BA 11), the left precuneus (BA 7), the left inferior parietal lobule (BA 40), and the right fusiform gyrus (BA 19) (Table 2).

The main effect of time lag resulting from the 2 (time lag) - by - 2 (memory task) ANOVA *without* the covariate “difference in the number of correctly Remembered word pairs between the 24 h lag and the 10 min lag added over both

memory tasks" yielded activity differences in the same brain areas as the ANCOVA. Only one additional activation site appeared in the comparison of the 10 min retrieval versus the 24 h retrieval within the left superior frontal gyrus (-18, 42, 38; BA 9).

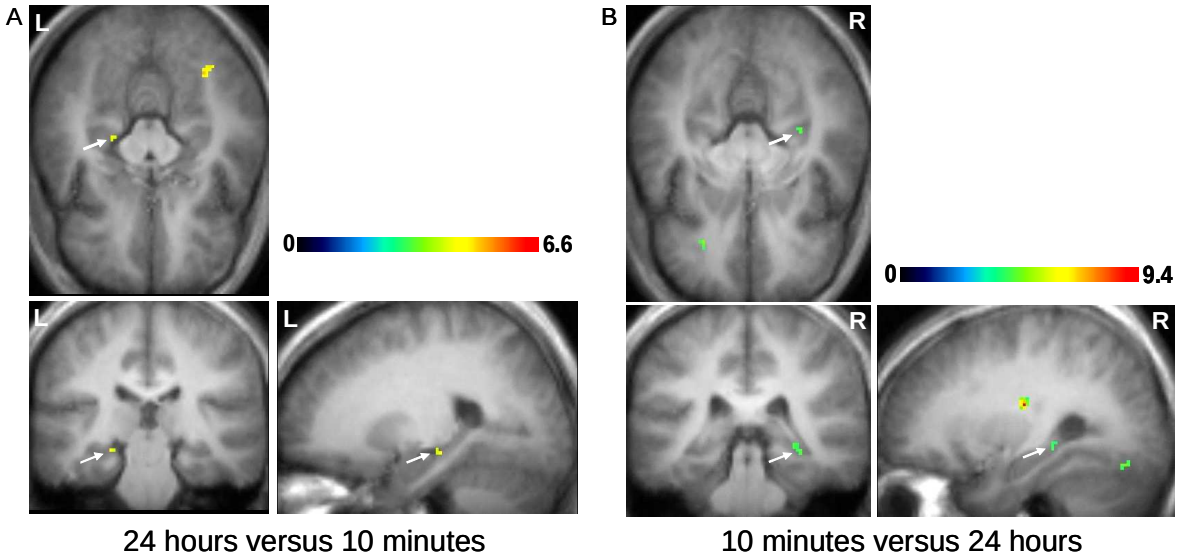

Figure 3. Time lag related activity differences in the MTL. A: Enhanced activity in the left hippocampal formation (arrows) during retrieval at the 24 h versus the 10 min lag. B: Enhanced activity in the right posterior hippocampal formation (arrows) during retrieval at the 10 min versus the 24 h lag. Results are shown in color-coded T values representing the main effect of 'time lag' in a two (time lag) - by - two (memory task) ANCOVA. T-maps are superimposed on the averaged and spatially normalized anatomical MR scans of all participants. R, right side of brain; L, left side of brain.

One-way ANCOVA

Time lag effects within the MTL for each separate task were revealed in the anterior right hippocampal formation (30, -16, -26) for the high-associative task and in the right anterior perirhinal cortex (36, -4, -32) for the low-associative task in the comparison of the 24 h lag versus the 10 min lag with the covariate "difference in the number of correctly Remembered word pairs between the 24 h lag and the 10 min lag". The reversed contrasts (10 min lag versus 24 h lag) yielded one MTL area of activity change in the right posterior hippocampal formation (34, -36, -4) for the high-associative task. The location of this right posterior hippocampal activity difference corresponded to the location of the right posterior hippocampal activity difference found in the main effect of time lag of the ANCOVA.

Conjunction analyses

Enhanced activity at the 24 h retrieval compared to the 10 min retrieval shared by both tasks was situated in the right fusiform gyrus (BA 19) (Table 2). There was a tendency ($p < 0.005$) towards an activity increase in the left hippocampal formation (-26 -24 -15) and the right perirhinal cortex (26 -24 -29). Conversely, enhanced activity at the 10 min retrieval compared to the 24 h retrieval shared by both tasks was located in the right posterior hippocampal formation (Table 1), the left middle frontal gyrus (BA 9) (Table 2), the left inferior frontal gyrus (BA 45, 47), the right inferior parietal lobule (BA 40), and the right fusiform gyrus (BA 19).

Correlation of the fMRI signal with retrieval performance

Retrieval Quantity (hits plus correct rejections in %): For the 24 h lag and the *high-associative task,* there was a positive correlation between the retrieval contrast (retrieval vs. baseline condition) and concurrent behavior (retrieval quantity) situated in the left (Fig. 5A) and right (Fig. 5B) anterior hippocampal formation (Table 1) as well as in many bilateral prefrontal (BA 9, 10, 46), temporal (BA 20, 22, 37), and parieto-occipital (BA 7, 17, 19) areas. For the 10 min lag and an equal statistical threshold ($p = 0.001$), this correlation failed to yield significant results in the MTL and reached significance at fewer neocortical sites than seen at the 24 h lag (BA 8, 9, 17, 31). Nevertheless, at a threshold of $p = 0.01$, this correlation yielded also an effect at the 10 min lag in the left anterior MTL, namely in left perirhinal cortex (-30, -12, -32).

For the 24 h lag and the *low-associative task,* there was a positive correlation between the retrieval contrast (retrieval vs. baseline condition) and concurrent behavior (retrieval quantity) located in the left perirhinal cortex (Table 1), the left (BA 6) and right (BA 9/10) middle frontal gyrus and the left anterior cingulate gyrus (BA 32). For the 10 min lag and an equal statistical threshold ($p = 0.001$), this correlation failed to yield significant results in the MTL but reached significance at neocortical sites (BA 6, 9, 18, 22, 47). Again, when the statistical threshold was lowered to $p = 0.01$, this correlation yielded also an effect at the 10 min lag in the left parahippocampal cortex (-26, -32, -22).

Retrieval Quality (# correctly Remembered word pairs): For the 24 h lag and the *high-associative task,* there was a positive correlation between the retrieval contrast (retrieval vs. baseline condition) and concurrent behavior (retrieval quality) situated in

the left anterior hippocampal formation and left entorhinal cortex (Table 1) as well as in neocortical Brodmann areas 9, 19, 32, 37, 40, 46. For the 10 min lag and an equal statistical threshold (p = 0.001), this correlation yielded significance in only one voxel of the MTL within the left anterior hippocampal formation (-34, -22, -12) as well as in neocortical Brodmann areas 4, 7, 8, 19, 21, 32, 45.

For the 24 h lag and the *low-associative task,* the correlation between the retrieval contrast (retrieval vs. baseline condition) and concurrent behavior (retrieval quality) yielded no significant effects (p = 0.001) in the MTL but in neocortical Brodmann areas 4, 7, 40, 41. Equally, for the 10 min lag and the same statistical threshold (p = 0.001), this correlation yielded no MTL effects but only neocortical effects in Brodmann areas 4, 7, 18, 22, 44.

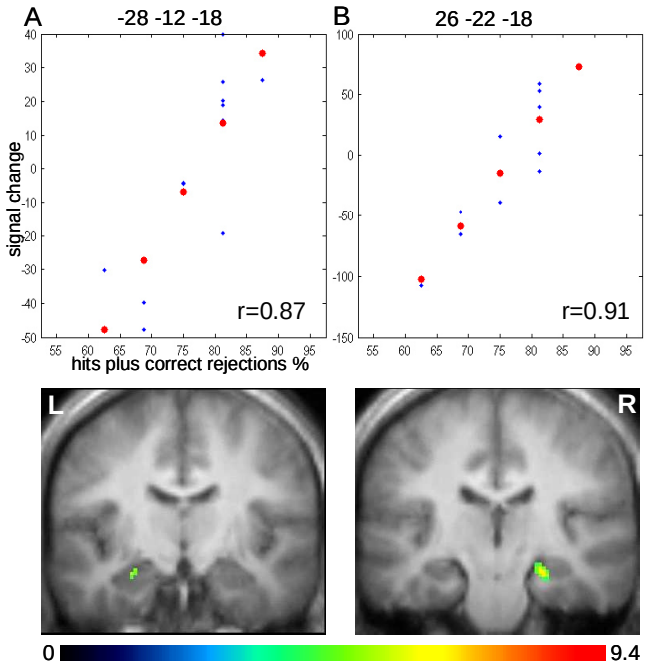

Figure 4. Correlation of fMRI signal with concurrent behavior. The displayed correlations are between retrieval contrasts (retrieval vs. visual baseline condition) and retrieval quantity (hits plus correct rejections in %) at the 24 h lag for the high-associative task. Significance was yielded in the left (A; r = 0.87) and right (B; r = 0.91) hippocampal formation at the indicated MNI coordinate positions. Red points indicate the regression line, blue points stand for the individual subjects. The corresponding T maps (SPM99) are presented below the scatter plots superimposed on the averaged and spatially normalized anatomical MR scans of all participants. Correlation strength is given in color-coded T values. R, right side of brain.

DISCUSSION

We examined retrieval-related brain activity with BOLD fMRI at 10 min and 24 h after incidental learning to test for effects of memory consolidation on brain activation in a sample of 12 high performing adults. Upon debriefing, none of the participants reported to have expected a memory test or to have intentionally rehearsed the learning material between study and test. The comparison of brain activity in two instead of just one memory task allowed us to look at commonalities between tasks rather than task-specific effects. We used more effective learning methods for the 24 h retrieval than the 10 min retrieval to yield a comparable retrieval quantity (hits plus correct rejections) and retrieval quality (Remember/Know) (Tulving, 1985) between the two time lags per task. Unfortunately, the learning strategy used for the 24 h retrieval in the high-associative task was so effective that the retrieval quality became almost significantly better at the 24 h than the 10 min lag. This effect was paralleled by significantly faster correct answers given in the high-associative task after 24 h versus 10 min. Retrieval quality was empirically matched between time lags in the low-associative task and retrieval quantity was matched between time lags in both tasks. In view of the insufficient empirical control of retrieval quality in the high-associative task, we statistically controlled for retrieval quality in the fMRI data analysis by use of ANCOVA with the “difference in number of correctly Remembered word pairs between time lags” as a covariate.

The two (time lag) - by - two (memory task) ANCOVA revealed a main effect of time lag with enhanced left hippocampal and reduced right posterior hippocampal activity during the 24 h compared to the 10 min retrieval. The same voxels within the left hippocampal formation exhibited a tendency ($p < 0.005$) towards an activity increase at the 24 h versus the 10 min lag in the statistical conjunction of both tasks (with retrieval quality controlled) suggesting that the activity increase in the left hippocampal formation reflected a stabilization of memory traces over 24 h. The conjunction analysis also showed that the activity decrease in the right posterior hippocampal formation from 10 min to 24 h was an effect common to both tasks.

However, right posterior hippocampal activity was neither correlated with retrieval quantity nor retrieval quality at either time lag and for either task. This indicates that activity in the right posterior hippocampal formation was not related to the retrieval of word-word associations as required at test. Moreover, the activity at

this right posterior hippocampal site was most enhanced during the baseline condition which required participants to count angular-shaped consonants across consonant strings and to indicate the number of angular-shaped consonants by button press. This counting task apparently activated the right posterior hippocampal formation to a larger extent than either of the four retrieval tasks. Within the four retrieval tasks, activity was higher at the 10 min lags than the 24 h lags. A discussion of these right posterior hippocampal effects will follow.

Retrieval-related activity in the left anterior hippocampal formation not only exhibited a mean increase from the 10 min lag to the 24 h lag, but also strongly correlated with both retrieval quantity and retrieval quality in the high-associative task at the 24 h lag. These correlations were weaker at 10 min after learning. It therefore appears that the retrieval-related increase in left anterior hippocampal activity over the 24 h reflected progress in the stabilization of the semantic word-word associations and that the relationship between left anterior hippocampal activity and retrieval success became closer as consolidation progressed. Results were similar for the low-associative task where correlations were strong between left perirhinal activity and retrieval quantity at the 24 h lag but rather weak at the 10 min lag. We therefore conclude that the first 24 hours of consolidation are associated with a strengthening of the relationship between MTL activity and retrieval success.

Our finding of a consolidation effect for semantic word-word associations in the left anterior hippocampal formation converges with previous evidence. The left MTL has typically been activated during verbal as opposed to nonverbal memory processes (e.g., Kelley et al., 1998; Golby et al., 2001) and has been found necessary for verbal memory in patient studies (Milner, 1972). The left anterior rather than posterior hippocampal region has been found activated during the *semantic* encoding of single words (e.g., Wagner et al., 1998; Otten et al., 2001) and the learning of *semantic* word-word associations (e.g., Vandenberghe et al., 1996; Henke et al., 1999; Lepage et al., 2000a). Unfortunately, reports of retrieval-related hippocampal activation are more sparse (Cabeza and Nyberg, 2000) and the evidence for a retrieval-related anterior-posterior functional segregation within the MTL is equivocal (Lepage et al., 1998; Schacter and Wagner, 1999; Cabeza and Nyberg, 2000). Nevertheless, it appears that associative as opposed to single item learning/retrieval tends to activate anterior rather than posterior portions of the hippocampal formation (Henke et al., 1999; Schacter and Wagner, 1999; Sperling et

al., 2001, 2003; Jackson and Schacter, 2004). Moreover, our finding of a consolidation effect in the left anterior hippocampal formation for semantic word-word associations concurs with the topography of the neocortical connections with the MTL in the primate brain. Left anterior neocortical regions, which in the human include storage sites for lexical-semantic knowledge, are preferentially connected with left anterior parahippocampal and hippocampal sites (Insausti et al., 1987; Witter et al., 1989; Witter and Amaral, 1991; Suzuki and Amaral, 1994; Barbas and Blatt, 1995). These connections may provide left anterior hippocampal neurons access to the storage sites of lexical-semantic knowledge in temporo-frontal regions and seem essential for the formation and consolidation of new semantic word-word associations.

Next, we consider the implications of the observed activity decrease in the right posterior hippocampal formation from the 10 min to the 24 h lag. Unlike left anterior hippocampal activity, right posterior hippocampal activity was uncorrelated with retrieval success in either task and at either time lag. This lack of correlation indicates that the kind of information which had been retrieved over the right posterior hippocampus did not assist the retrieval of the word-word associations as required by our retrieval task. Instead, this posterior hippocampal area might serve the visual (word forms) rather than the semantic recognition of the individual words. Such a retrieval of visual information does not much assist the retrieval of associated word meanings as required by our retrieval task. This interpretation is supported by the observation that activity at this right posterior hippocampal location was highest during the baseline condition which required participants to count angular-shaped consonants across consonant strings. These consonant strings are devoid of meanings and are therefore unlikely learned as semantic entities. But it is conceivable that they were incidentally encoded with respect to their unusual visual appearance activating the same right posterior hippocampal area which showed some activity enhancement during the retrieval of recent (10 min) versus older (24 h) visual word information. The fact that this hippocampal site of differential activation was situated in the right rather than the left (Milner, 1972; Kelley et al., 1998; Golby et al., 2001) and the posterior rather than the anterior hippocampal formation supports the notion that activity in this area was related to the visual recognition of words (Schacter and Wagner, 1999) or to feelings of familiarity with words (Saykin et al., 1999; Strange et al., 1999) or the retrieval of perceptual aspects of words (Small et

al., 2001). Some kind of visual learning and retrieval underlying this right posterior hippocampal effect seems also likely when again considering the primate neuroanatomy. A heavier visual input is directed to the posterior than the anterior primate hippocampal formation (Insausti et al., 1987; Witter et al., 1989; Witter and Amaral, 1991; Suzuki and Amaral, 1994; Barbas and Blatt, 1995). The decay of right posterior hippocampal activity from the 10 min lag to the 24 h lag might correspond to the forgetting of visual word attributes and the increase in activity during the inspection of the consonant strings might correspond to the learning of the visual appearance of the novel configurations.

One might argue that a certain forgetting and associated weakening of memory traces over 24 h is just natural and should not be counteracted in a study of consolidation. Even worse, the control of retrieval quantity and quality between time lags induces the learning method as a possible confounding variable. Although the assimilation of retrieval performance by use of different learning methods seems desirable, effects of learning methods could bias the effects of consolidation. In spite of these obvious disadvantages, we prioritized a comparable retrieval quantity and quality between time lags. To alleviate potential effects of learning methods, we used two memory tasks and chose two different ways for the two tasks to equate retrieval performance between time lags. Depending on the task, either the learning strategy or the number of learning runs remained constant between time lags. These two ways of improving learning have been shown to induce separate activation patterns within the MTL (see Heckers et al., 2002). Therefore, the statistical conjunction, which tested for effects shared by both memory tasks, likely generated effects of time lag rather than learning method. Moreover, the fact that in each memory task correlations between the medial temporal fMRI signal and retrieval success became stronger as time went by, speaks in favor of an effect of time lag rather than an effect of learning method.

It should be noted that some of the participants reported that they had become aware that a big portion of the word pairs presented for retrieval were Old. Nevertheless, these participants ensured that they had based their Old/New decisions on their ability to retrieve associations and not on their personal probability estimates. This assertion is supported by the correlation strength between the hippocampal/perirhinal fMRI signal and retrieval performance measured in hits plus correct rejections. In addition, the second performance measure was Remember

versus Know (Tulving, 1985) which is independent of a possible tendency to give Old responses and which correlated also with the hippocampal fMRI signal in the high-associative task.

Regarding the neocortical contributions to the retrieval of word pairs, we noted a general trend towards less retrieval-related neocortical activity at 24 h than 10 min after learning. This may suggest that the stabilization of memory traces within the neocortex coincided with a more efficient use of the retrieval network. Specifically, the comparison of the 10 min retrieval with the 24 h retrieval revealed greater activity in the left prefrontal cortex (BA 9, 11, 45, 47), the left precuneus (BA 7), and bilateral sites of the inferior parietal lobule (BA 40). Conversely, the comparison of the 24 h retrieval with the 10 min retrieval yielded an activity enhancement in the left inferior temporal gyrus (BA 37) and the right frontal pole (BA 10). Many of these prefrontal activity differences are located in areas which have been consistently observed in imaging studies of memory retrieval (Lepage et al., 2000b; Buckner, 2003). The left hemisphere areas 45 and 47, which exhibited greater retrieval-related activity at 10 min versus 24 h, are generally believed to become activated when new and rather weak associations must be recovered at the exclusion of other possible, but situationally-inappropriate, associations (Buckner, 2003). Accordingly, the enhanced level of activity in areas 45 and 47 may indicate a greater demand for selection of appropriate representations when the memory traces are still fragile. This prefrontal selection of memory representations works through interactions with posterior multimodal association cortices including the temporal neocortex, precuneus and the inferior parietal lobule which also exhibited differential activity between time lags. Remarkably, the prefrontal region most strongly associated with episodic memory retrieval in the literature, the right frontal-polar region (BA 10), exhibited increased activity during the 24 h retrieval compared to the 10 min retrieval; activity in this region is associated with the maintenance of an episodic memory retrieval mode (REMO) (Tulving et al. 1994; Cabeza and Nyberg, 2000; Lepage et al., 2000b; Buckner, 2003). REMO refers to the state in which attention is directed to a segment of one's personal past for potential retrieval (Lepage et al., 2000b). It thus appears that the maintenance of an episodic memory retrieval mode was more warranted for the retrieval of 24 h old than 10 min old memories.

Finally it should be mentioned that the IQ and memory scores of our subject sample were unusually high which could potentially limit the external validity of our

findings. A selection bias towards high performing individuals might have occurred due to the locations where participants had been recruited (advertisements at the University of Zurich and at a Swiss bank).

In conclusion, our fMRI results in the human suggest that the consolidation of semantic word-word associations over 24 h was associated with an increase in retrieval-related hippocampal activity and a strengthening of the relation between hippocampal activity and retrieval success. Although fMRI is only an indirect measure for excitatory presynaptic and postsynaptic processing, our fMRI findings of an increase in hippocampal activity and a stronger correlation between retrieval success and hippocampal activity after a day than 10 min are in agreement with the neurobiological indications in animals of a strengthening of hippocampal synaptic connections and a growth of new synaptic connections within the first 24 h after learning.

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1 month of memory consolidation enhances retrieval-related hippocampal activity

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ABSTRACT

Most studies on system consolidation investigate whether there is reorganization in the storage of autobiographical memories that go back years or even decades. The effects of long-term memory consolidation on retrieval related brain activity in a time range of days and months have hardly been examined so far.

We compared cerebral activations during the retrieval of word pairs learned 1 day versus 1 month ago in 12 good learners and 8 poor learners. A prospective design allowed us to control those variables which had confounded other neuroimaging studies of memory consolidation: nature of encoding, rehearsal between study and test, retrieval quantity and quality. Recognition was measured adding the Remember/Know paradigm to ascertain the number of “old” responses due to episodic recollection versus feelings of familiarity. We administered a low-effective (2 runs) and a high-effective (6 runs) incidental learning procedure to two word lists each which were either retrieved after 1 day or 1 month. In good learners, the high-effective learning procedure yielded an equal retrieval performance (% correctly Remembered - % falsely Remembered) after 1 month as the low-effective learning procedure after 1 day. Poor learners did not benefit from the high-effective learning procedure, so that retrieval quality could not be equalized across time-lags. Good learners exhibited enhanced retrieval-related hippocampal activity 1 month vs. 1 day following learning. This result was obtained irrespective of whether the nature of learning was kept constant (and retrieval performance varied between time-lags) or retrieval performance was kept constant (and the nature of learning varied between time-lags). Poor learners, on the other hand, exhibited decreased retrieval-related hippocampal activity 1 month vs. 1 day after learning reflecting their dramatic decrease in retrieval performance over 1 month. Over the whole group, retrieval-related bilateral hippocampal activity at the 1-day-lag predicted the amount of forgotten word pairs after 1 month: the greater hippocampal activity during the 1-day-retrieval, the larger the decrease in retrieval performance from 1 day to 1 month. The observed increase in MTL activity during the 1-month-retrieval may be evidence for a proliferation and spreading of memory traces in the MTL during the process of condolidation as predicted by the multiple trace theory. According to the correlation analysis, the increase might alternatively be interpreted as an enhanced recruitment of retrieval-related neural structures when task demands are high.

INTRODUCTION

The findings that patients with medial temporal lobe (MTL) lesions were able to retrieve remote information whereas recent information was lost, formed the foundation for the theory that medial temporal structures are critical for memory formation and storage over a limited time only and that after a period of consolidation neocortical structures are sufficient for the retrieval of remote memories (Squire & Alvarez, 1995). This often termed standard model of memory consolidation was also supported by prospective animal studies that allowed more careful control over lesion sizes and locations (Squire, Clark, & Knowlton, 2001).

In contrast, the multiple trace theory (MTT) (Nadel & Moscovitch, 1997), proposes that the hippocampus is continuously involved in the storage and retrieval of episodic information. According to the MTT an experienced episode is stored as a memory trace that consists of a bound ensemble of hippocampal and neocortical neurons. Over time and upon reactivation of the episode or parts of it, multiple memory traces are formed and dispersed over wider areas of the hippocampal formation. This multiple representation of older memories renders them less susceptible to disruption by hippocampal lesions. The MTT provides an alternative explanation for the temporally graded patterns in retrograde amnesia and can also account for completely flat gradients that were observed after restricted MTL lesions (Nadel & Moscovitch, 1997; Nadel, Samsonovich, Ryan, & Moscovitch, 2000; Rosenbaum, Winocur, & Moscovitch, 2001). In contrast to the standard model which suggests an equal involvement of the hippocampus in fact and event-specific memory, only true episodic memory is claimed to depend permanently on the hippocampal formation. General semantics, as for example the conceptual knowledge about words and objects, are believed to become eventually independent from the MTL and to rely then on neocortical structures exclusively. The model predicts that the higher the episodicity of a memory, the more permanent is its reliance on the MTL.

Unfortunately, in animals such fine distinctions between different memory types are not possible. On the other hand, studies in patients have the disadvantage that the extent of medial temporal lesions and damage to structures beyond the medial temporal lobe cannot always be reliably determined (Eichenbaum, 2001). Neuroimaging studies offer the opportunity to identify the relevant structures that play a role in memory consolidation in healthy participants and allow a reliable assessment of the type of information that is retrieved. In a neuroimaging study

comparing recent and remote memories, the standard model would predict a decrease of hippocampal activity with increasing age of memory. The MTT would predict equal activity levels in the MTL during the retrieval of recent and remote information as long as the compared memories have the same episodic quality.

Yet, the imaging studies that compared recent and remote memory are equivocal. While some (Haist, 2001; Niki & Luo, 2002; Tsukiura et al., 2002; Piefke, Weiss, Zilles, Markowitsch, & Fink, 2003) did find temporally graded activity patterns in the medial temporal lobe, others (Maguire, Henson, Mummery, & Frith, 2001; Ryan et al., 2001; Maguire & Frith, 2003) did not. The reason for the inconsistency may lie in the differences regarding the methodological approaches. For example memory quality was not controlled in all studies. Regarding the dissociation of episodic and semantic memory as proposed by Nadel and Moscovitch (1997), it seems crucial to focus on episodic memory only.

In general the retrospective designs used bear a number of problems. There is no information about how well or how fast and in which context the memory was acquired. Unequal learning events could lead to differences in storage and retrieval (Murray & Bussey, 2001) and affect brain activity during retrieval in an uncontrollable way. Second, in most studies, retrieval performance was not assessed directly, but in a post-scan evaluation. However, a direct behavioural measure that corresponds to the measured brain activity is the only way to ensure that brain activities do not merely reflect performance differences instead of consolidation effects.

Most of the above cited studies, using a cued recall design, assessed autobiographical memories prior to scanning. However, this procedure has the disadvantage that the memories are reactivated during the interview. Although not yet completely established, several lines of evidence suggest that reactivation of a stable memory may render it labile and sensitive for disruption or modification again (for a review see Nader, 2003). Importantly this phenomenon has not only been observed for cellular but also for system consolidation. Debiec, LeDoux, & Nader (2002) showed that intra-hippocampal infusions of a protein-synthesis inhibitor caused amnesia for a consolidated contextual fear memory, but only if the memory was reactivated prior to infusion. From this point of view, a reactivation of a particular memory by an interview prior to scanning may modify the memory in a way that at least parts of the memory have changed or were replaced by new traces (Nadel & Land, 2000; Nader, Schafe, & LeDoux, 2000). Hence, the age of the traces that

contribute to the memory of the particular episode may not be the same as prior to reactivation. In other words, it may well be that the measured memories during scanning were at least partly related to the interviewing episode and not solely to the time the episode was originally encoded.

Only a prospective design allows proper controlling for the factors that are often confounded in neuroimaging consolidation studies, such as amount and quality of retrieval, encoding procedure, and rehearsal in the time between study and test. One study so far by Stark and Squire (2000) chose this approach to compare memory associated brain activities during retrieval of line drawings one-half hour, 1 day and one week after learning. Since no effect of study-test interval was observed the authors concluded that the time span was too short to observe changes in activity.

We compared retrieval-associated cerebral activations of 1 day and 1 month following learning in 12 good and 8 poor learners and were interested in the following questions: 1) How does retrieval-related activity differ between the two retrieval time-lags? 2) What are the relationships between brain activity and retrieval performance? 4) Can brain activities during the 1-day-retrieval already predict performance after 1 month?

Learning of word pairs took place with a set method and precise instructions either 1 month or 1 day before retrieval, which was all measured during a single fMRI session (Fig. 1). Participants did not know that they later had to retrieve all learned material. Retrieval performance was assessed with the Remember/Know paradigm to ascertain that true episodic recollection and not only retrieval based on feelings of familiarity was measured. We administered a low-effective (2 runs) and a high-effective (6 runs) learning procedure during each of the two learning sessions. The 1-month-retrieval after the high-effective learning procedure resulted in equal retrieval performance (% correctly Remembered - % falsely Remembered) as the 1-day-retrieval of word pairs learned with the low-effective (2 runs) learning procedure. Thus, we could not only compare the fMRI signal during 1-month- versus 1-day-retrieval when learning procedures were equal, but also when retrieval performance was equal. A questionnaire administered upon debriefing allowed excluding those two participants from the analysis who did rehearse the material in the time between study and test.

MATERIALS AND METHODS

Participants

24 healthy students (8 men and 16 women) aged 20-31 years (mean: 24.9, SD: 2.7) volunteered for this study. Informed written consent was obtained after the nature and possible consequences of the fMRI study were explained. Four participants were excluded due to inappropriate learning or rehearsal between study and test.

Stimuli

In each learning and retrieval condition, 12 pairs of unrelated abstract nouns (Henke, Weber, Kneifel, Wieser, & Buck, 1999) were presented. Word lists were counterbalanced over conditions. In the baseline condition, stimuli consisted of 12 pairs of unpronounceable consonant strings which had an equal average length as the abstract nouns. The sequence of consonants per string was randomly generated.

Memory tasks

General procedure. The experiment consisted of a learning run on day 1, a second learning run 27 days later on day 28 and the retrieval of all learning material on the following day on day 29. Thus, half the stimuli were retrieved 1 month after learning and half 1 day after learning. Because a natural forgetting is taking place during the delay period of 1 month, we also included a learning procedure which was very effective and led to a similar retrieval performance after 1 month and 1 day. For more effective learning, participants performed six instead of just two learning runs on a set of word pairs. Thus two sets of word pairs were learned on day 1: a set with two and a set with six learning runs. To equate the number of learning runs, we also included a six-fold learning task on day 28. In addition, a two-fold learning of a fourth set of word pairs was conducted on day 28. The retrieval performance of word pairs learned six-fold on day 1 was expected to correspond to the retrieval performance of word pairs learned two-fold on day 28 (Fig. 1a). Thus, this procedure allowed the comparison of retrieval-related brain activity of 1-day- and 1-month-retrieval with both equalized learning parameters and equalized retrieval performance. Numbers of learning runs had been tested in pilot experiments.

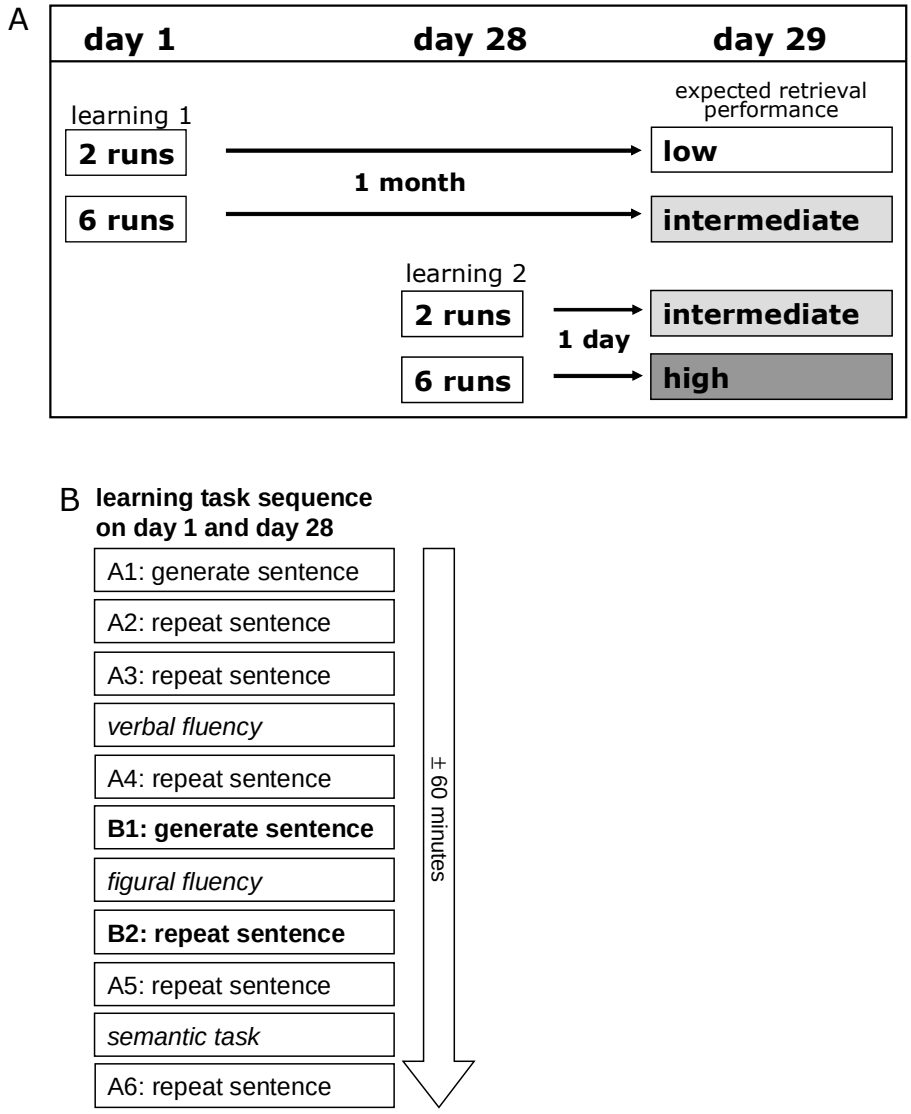

Figure 1. Task procedure (A) and task sequence (B). A: There was a learning session on day one and a second learning session (with different learning material) on day 28. A: Retrieval of all learning material was carried out on day 29, i.e., 1 month after the first and 1 day after the second learning session. In each learning session two lists of 12 word pairs had to be learned, one with 2 runs, the other with 6 runs. The retrieval instruction was to decide whether a word pair was 'Remembered', 'Known', or 'New'. A visual task was included as a baseline condition in the retrieval fMRI time-series with the instruction to count the consonants that were as high as capitals across pairs of consonant strings. B: Learning instructions were to generate a sentence which contained both words of a pair and repeat the sentences in the following runs. The learning runs alternated with semantic distracter tasks. The distracter tasks were included to induce short delay times between the individual learning runs and therefore a progressively deeper encoding.

Learning on day 1 and 28. Two sets of word pairs were learned on day 1 and another two sets on day 28. All learning conditions consisted of incidental paired-associate word learning. Participants were presented with the 12 word pairs of one set, one pair at a time. The learning instruction was to generate a meaningful sentence out loud which contained both words of a pair. Subsequently, the same sentences had to be repeated upon renewed presentation of the word pairs. There was no time-limit for generating and repeating the sentences. The two learning sets were presented in an interleaved manner. In addition, between some of the learning runs, participants had to complete a verbal fluency test, a figural fluency test and a semantic memory test. These tasks were interspersed as distracter tasks to induce a progressively deeper encoding than can be expected with immediately repeated learning runs. The sequence of tasks was the same for all participants and for both days (Fig. 1b): 1. Generate the sentences for 12 word pairs of the six-run-learning condition and repeat the same sentences upon renewed presentation of the word pairs immediately twice. 2. Do the verbal fluency task. 3. Again repeat the sentences of the six-run-learning condition. 4. Generate the sentences for 12 word pairs of the two-run-learning condition. 5. Do the figural fluency task. 6. Repeat the sentences of the two-run-learning condition once. 7. Repeat the sentences of the six-run-learning condition. 8. Do the semantic memory task. 9. Repeat again the sentences of the six-run-learning condition.

Distracter tasks. The distracter tasks were presented interleaved with the two-fold and the six-fold learning conditions on both days. The distracter tasks were simple but stressful because of time limits. They should prevent participants from rehearsing the generated sentences. For the verbal fluency tasks participants had to generate over three minutes adjectives beginning with the letter ‘a’ in the learning session on day 1, and verbs beginning with the letter ‘s’ in the learning session on day 28. The figural fluency task was in both sessions the five point figural fluency task during one minute. The semantic memory task was to name during one minute if possible four members of each of the following categories: brand of German cars, shower gels, African cities, famous painters, and American actors in the first learning session and toothpastes, Arabic countries, composers, European Actresses and Formula-One drivers in the second learning session.

Retrieval tasks. On day 29, the retrieval of all learning material was assessed with a recognition test that contained 75% ‘old’ word pairs (pairs presented for

learning) and 25% ‘new’ word pairs composed of old words (presented for learning) that were recombined. Participants were required to answer with ‘remember’ if they could recollect the episode of generating a sentence with the two words, ‘know’ if they knew that the words were presented together during learning but without recollecting the episode, or ‘new’ if they felt that the word pair was a new combination of old words. The task was the same for all retrieval conditions. Retrieval conditions only differed in the time delay between learning and retrieval (1 month vs. 1 day) and the number of learning runs applied on day 1 and day 28 (two runs vs. six runs), resulting in a 2 x 2 factorial design. To have the participants think back to the correct encoding time period, instruction slides preceding the retrieval conditions indicated whether the word pairs were learned on day 1 or day 28. The mental travel back to the correct encoding time period prevented memories from the wrong set or time period.

We also included a visual baseline task (5^{th} condition) which required a low-level visual analysis of pairs of unpronounceable consonant strings. The instruction was to count all consonants that had the same height as capitals (e.g. h, t, b, vs. c, n, w) across the two strings and indicate the number by button press (left button for less than eight, right button for eight or more). Consonant strings were chosen for this task because they neither contain meaning nor bear a phonological resemblance to real words excluding the encoding of word meaning in this baseline condition.

At the end of the retrieval session, participants were explicitly asked whether they repeated the learned sentences during the intervening month between study and test and whether they gave their answers according to an expected pattern of ‘old’ word pairs. Participants that answered one of these questions with ‘yes’ were excluded from further analysis.

fMRI design

fMRI data were collected during the retrieval of all four sets of word pairs and the baseline task in one time-series with blocked trials, which lasted 6 min and 15 s. The time-series consisted of 15 epochs, 3 epochs for each of the 5 conditions. Each epoch lasted 25 s and comprised the presentation of 4 stimuli (6 s per stimulus) and an instruction slide displayed for 1 s at the beginning of each epoch (Fig. 2). Epochs were regularly alternating in a fixed order. The sequence of conditions was counterbalanced over participants. Each retrieval epoch consisted of three old and

one new word pair. The sequential position of the new word pair in the stimulus sequence of an epoch was randomly assigned across epochs.

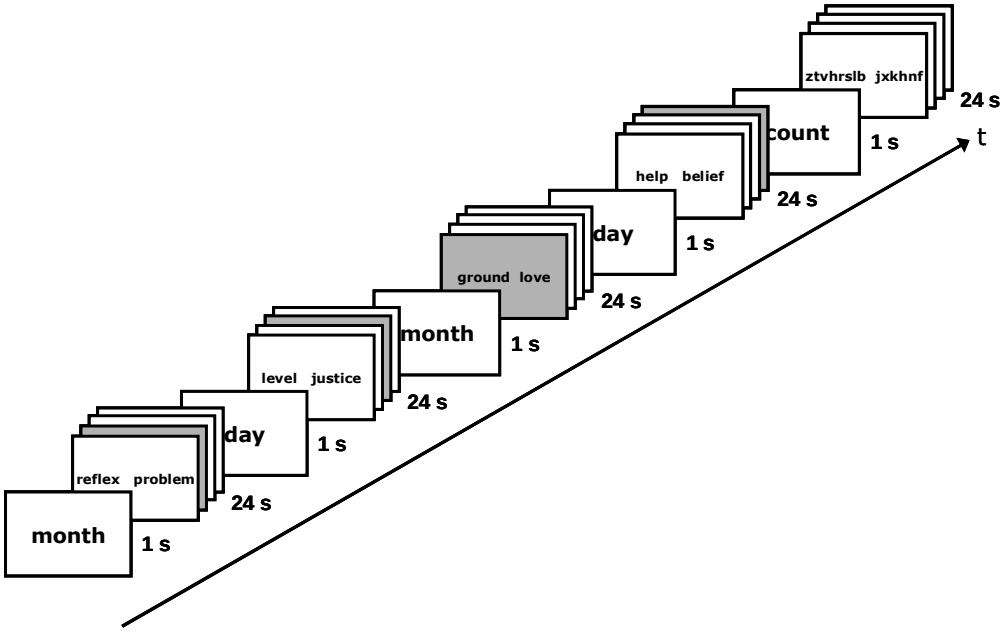

Figure 2. The retrieval fMRI time-series included blocked trials of the four retrieval conditions and the visual baseline condition. There were three epochs per condition (the illustration shows only one task cycle). One out of the four trials per retrieval epoch contained a new word pair (grey shaded for illustration). The first slide of each epoch was an instruction slide which indicated either the time lag for retrieval or the instruction to count.

Stimulus presentation was performed with the software tool SCOPE (Max Duersteler, University Hospital Zurich, Switzerland). The computer-driven stimulation (640x480 resolution, 60 Hz refresh rate, 32-bit color depth) was projected with an LCD-projector to the back of a screen that participants could watch through a mirror which was attached to the head coil of the MR scanner. Participants' responses were recorded via a fiber-optic response pad with a four in-line button hand-held box (Current Designs, Inc., Philadelphia, USA).

Image acquisition and post-processing

We used the fast imaging technique Sensitivity Encoding (SENSE) (Pruessmann, Weiger, Scheidegger, & Boesiger, 1999; Weiger, Pruessmann, & Boesiger, 2002) on a 3 Tesla MR Imaging system (Philips Medical Systems Inc, Best, The Netherlands) equipped with a transmit-receive body coil and a commercial eight-element head coil array (MRI Devices Corporation, Waukesha WI, USA). Parallel

imaging techniques like SENSE provide enhanced encoding efficiency which is achieved by using spatially varying coil sensitivity profiles, allowing reconstruction of under-sampled data. The benefits are a faster data acquisition, a reduction of susceptibility artifacts, distortions and blurring and a substantial increase in spatial resolution (Preibisch et al., 2003). This also improves anatomical assignment of activations and facilitates transformation to standard brain templates. The drawback, however, is a slightly reduced signal-to-noise-ratio (SNR).

Functional T2*-weighted images were acquired from 32 axial slices covering the whole brain with an acquisition matrix of 80x80 (voxel size 2.75 x 2.75 x 4 mm^3) and were reconstructed to an image matrix size of 128 x 128. A SENSE single-shot-echo-planar-imaging (SENSE-sshEPI) readout was applied with a reduction factor of 2.0, corresponding to an acquisition bandwidth of 34.8 Hz. Further acquisition parameters were TE = 35 ms, TR = 3000 ms, flip angle 82°, slice thickness 4 mm, no slice gap. A SENSE-reference scan for sensitivity calibration used for SENSE data reconstruction was acquired for each volunteer with 3.75 x 3.75 x 3.75 mm3 spatial resolution, acquisition matrix 64 x 64, shortest TE, shortest TR, flip angle = 7°.

Image post-processing and the statistical analysis were performed using Statistical Parametric Mapping (SPM2b; http://www.fil.ion.ucl.ac.uk/spm/). Volumes were realigned to the first volume to remove movement-related variance components. As a prerequisite for inter-participant averaging and to facilitate the reporting of results, images from all participants were spatially normalized into standard stereotaxic space (standard EPI template SPM2b) (Friston, Ashburner, Frith, Heather, & Frackowiak, 1995). Data were smoothed to a full width of 8 mm at half-maximal resolution using a Gaussian filter to increase signal to noise ratio and to conform data to a Gaussian field model.

Statistical analysis of fMRI data

The fMRI data of all valid participants (n = 20) were analyzed voxel by voxel modeling the conditions as box car functions convolved with a hemodynamic response function and applying the general linear model (fixed effects model) (Cohen, 1997) provided in SPM2b. A high-pass filter with a cut-off period of 128 s was used. The six head movement parameters were included as confounding factors. First level linear contrasts for each subject were taken to the second level. Group analyses were conducted using random-effects analyses, analyses of

covariance and correlation analyses. A threshold of $p<0.001$ was applied and significant activation clusters of more than three contiguous voxels were interpreted.

Activity differences between time lags in good learners (n = 12).

1. Empirically equalized performance due to different learning procedure: The 1-month-retrieval of word pairs learned with six runs was compared to the 1-day-retrieval of word pairs learned with two runs in a fixed-effects model for each participant. The resultant within-subject effects were then tested against zero in a second level random effects analysis (SPM2b, basic models).
2. Statistically controlled retrieval performance with equal learning procedure: The 1-month-retrieval of word pairs learned with six runs was compared to the 1-day-retrieval of word pairs learned with six runs in a fixed-effects model for each participant. The resultant within-subject effects were then entered in an analysis of covariance with retrieval performance (% correctly Remembered word pairs - % falsely Remembered word pairs) as nuisance variable in a second level random effects analysis (SPM2b, basic models).
3. Equal learning procedure but different performance: The 1-month-retrieval of word pairs learned with six runs was compared to the 1-day-retrieval of word pairs learned with six runs in a fixed-effects model for each participant. The resultant within-subject effects were then tested against zero in a second level random effects analysis (SPM2b, basic models).

Activity differences between time lags in poor learners (n = 8)

In the poor learners group the same analyses as in the good learners were conducted except for step 1 because the poor learners had no empirically equalized performance.

Correlation of the fMRI signal with retrieval performance (n = 20).

Correlations refer all to the retrieval after learning with 6 runs.

1. To assess the relationship between brain activity and retrieval performance and reaction time for correctly Remembered word pairs at the 1-month- and the 1-day-lag, we computed fixed-effects analyses comparing each retrieval condition with the baseline condition for each participant and then correlating the resultant within-subject effects with the retrieval performance and reaction time of the respective condition (% correctly Remembered - % falsely Remembered).

2. To assess the predictive value of activation during the 1-day-retrieval for retrieval performance after 1 month, we correlated the within-subject contrasts of the 1-day-retrieval vs. baseline with retrieval performance (% correctly Remembered - % falsely Remembered) after 1 month.
3. To assess the predictive value of activation during the 1-day-retrieval for the amount of information that gets lost over the time delay of 1 month, we correlated the within-subject contrast of the 1-day-retrieval vs. baseline with the retrieval performance difference between 1 day and 1 month (day (% correctly Remembered - % falsely Remembered) – month (% correctly Remembered - % falsely Remembered)).

RESULTS

Behavioral data

Retrieval performance was calculated as the percent old word pairs that received a remember response minus the percent new word pairs that received a remember response (% correctly Remembered - % falsely Remembered). Twelve participants reached the criterion of a 50% retrieval performance in all four retrieval conditions except for the retrieval after the 2-run-learning condition and a month's delay and were assigned to the good learners group. Of the remaining 10 participants, two were excluded because they retrieved much more word pairs at the 1-month-lag compared to the 1-day-lag suggesting that they did not properly encode the word pairs during the second learning session. Thus, the poor learners group consisted of 8 participants. The whole sample which was used for the correlation analyses consisted of 20 participants.

The good learners showed an equally high mean retrieval performance (mean ± SD) for the retrieval of word pairs learned with 6 runs 1 month ago (69.4 ± 15.8) and word pairs learned with two runs 1 day ago (70.3 ± 9.8); ($t(11) = -0.15$; $p = 0.884$). Equal performance was also found for reaction times (mean in ms ± SD) for retrieval of word pairs learned with 6 runs 1 month ago (3224 ± 700) and word pairs learned with two runs 1 day ago (3060 ± 760); ($t(11) = 0.64$; $p = 0.536$). Due to the expected natural decay of memory, retrieval of word pairs learned 1 day ago was significantly better than retrieval of word pairs learned with the same number of learning runs 1 month ago. This was especially true for the two-run-condition – day

(70.4 ± 9.9) versus month (30.6 ± 17.2); (t (11) = -6.3; p = 0.000) – but also for the six-run-condition – day (87.0 ± 10.4) vs. month (69.4 ± 15.8); (t (11) = -4.4; p = 0.001) (Fig. 3a).

The poor learners (27.8 ± 13.3) significantly differed from the good learners (69.4 ± 15.8) solely in the retrieval of word pairs learned with 6 runs 1 month ago (Z (19) = -3.7; p = 0.000) (Fig. 3a). All of the subjects in the good learners group profited from the 6 learning runs for retention over 1 month whereas the loss of information was high despite the high number of learning runs in the poor learners group (fig 3b). In fact, the poor learners' retrieval performance was equally low after 1 month in the two-run-learning condition (30.6 ± 27.1) and the six-run-learning condition (27.8 ± 13.3); (Z (7) = -0.43; p = 0.666) (fig 3a).

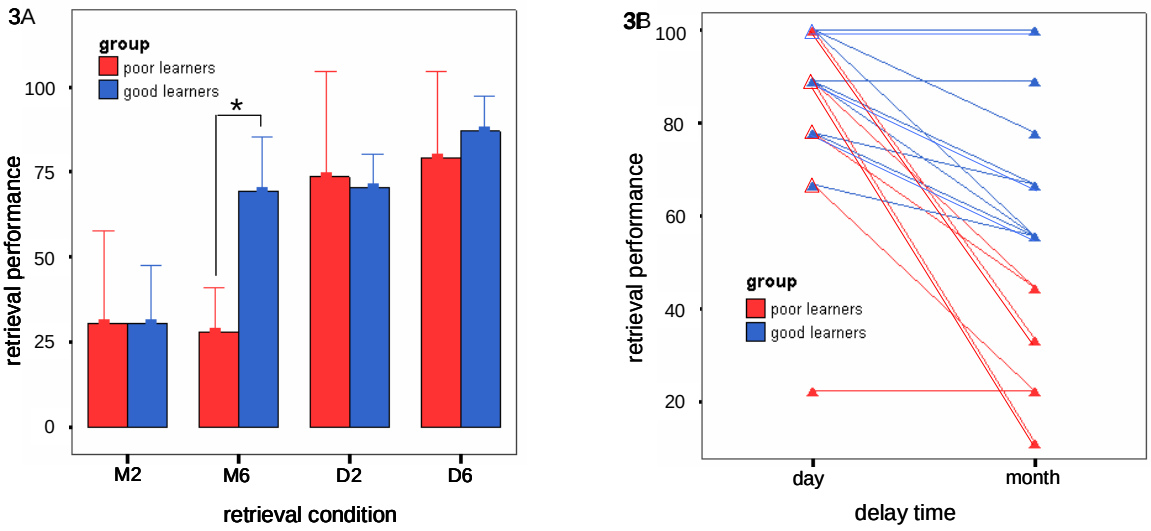

Figure 3. Mean (A) and single subject (B) retrieval performance (% correctly Remembered word pairs - % falsely Remembered word pairs) of good and poor learners. A: Good learners showed equal performance of word pairs learned with six runs 1 month before and word pairs learned with two runs 1 day before. Good learners show a significantly better retention of word pairs learned with six runs 1 month before than poor learners. Bars represent standard deviations. B: All good learners could keep their performance at a high level when retrieving word pairs learned with six runs after 1 day and 1 month. In all poor learners but one who showed very low performance already after 1 day, performance decreased dramatically after 1 month.

fMRI data

Activation differences between time lags in good learners (n = 12).

All three analyses to assess activation differences between retrieval after 1 month and 1 day showed that the memory retrieval network, including the MTL, left anterior temporal, fusiform and semantic frontal regions were more activated after 1 month than 1 day.

The direct comparison of the two retrieval conditions with an empirically equalized performance but different learning procedures (6 runs month vs. 2 runs day) revealed activations in bilateral parahippocampal, bilateral anterior temporal, bilateral frontal, bilateral cingular, and left fusiform areas (table 1).

The analysis of covariance in which differences in retrieval performance were partialled out statistically and the learning procedure was 6 runs for both conditions showed that the left and right hippocampus, left anterior temporal, left fusiform and left parietal areas were more active for 1 month than 1 day old memories (table 1). The activations in the left hippocampus, left anterior temporal, left fusiform, and left parietal areas and in addition in the left inferior frontal gyrus could also be observed in the same contrast without covariate (table 1).

In the day versus month contrasts of the retrieval conditions with equal learning (with and without covariate), no significant activations could be observed. The day versus month contrast of equal retrieval performance showed only the left cingulate gyrus (BA 24; -16, 0, 39; t = 4.7) was more activated for 1-day-old than 1-month-old memories.

Table 1. Brain regions showing significantly more activations during retrieval after one month than after one day.

Region of activation	equal learning procedure performance statistically controlled					equal learning procedure performance uncontrolled					different learning procedures performance empirically equalized				
	x	y	z	BA	T	x	y	z	BA	T	x	y	z	BA	T
R. hippocampus	20	-11	-20		4.6										
L. hippocampus	-30	-9	-21		5.6	-26	-30	-4		6.5					
						-30	-9	-21		5.0					
R. parahippocampal g.											22	-9	-21	28	5.1
L. parahippocampal g.											-11	-29	-2	35	5.0
											-20	-28	-9	28	4.9
L. superior temporal g.	-46	9	-6	22	6.2	-46	9	-6	22	6.1					
	-51	9	-14	22	4.9						-52	13	-12	38	5.0
R. superior temporal g.											51	17	-11	38	5.1
L. fusiform g.	-30	-88	-11	18	6.2	-30	-86	-9	18	4.9	-26	-76	-13	18	4.9
R. lingual g.						14	-80	-11	18	4.5					
L. inferior frontal g.						-26	21	-13	47	4.9	-44	17	-4	47	5.6
											-56	28	8	45	5.0
R. inferior frontal g.											53	31	-10	47	4.8
R. middle frontal g.											48	12	36	8	5.3
											-8	39	33	8	6.5
L. superior parietal g.	-24	-49	60	7	5.5	-24	-48	58	7	4.8					
R. cuneus						20	-69	26	17	5.8					
R. anterior cingulate											-4	21	28	24	4.9
L. anterior cingulate											16	36	13	24	6.4

R, right ; L, left ; BA, Brodmann area ; g, gyrus; x, y, z, Talairach coordinates;

Activation differences between time lags in poor learners (n = 8).

In the rather small group of eight poor learners, the analysis of covariance with 6 learning runs at both time lags revealed a pattern that seemed to be reverse to the good learners: several regions, including bilateral parahippocampal (BA 28, 30, 35), temporal (BA 20, 21, 22, 37, 39), bilateral fusiform and lingual (BA 18, 37), parietal (BA 5, 7) and left frontal (BA 9, 44, 45) areas, were more activated after 1 day than 1 month. The day versus month comparison without covariate revealed more or less the same activations in parahippocampal, temporal, parietal and fusiform and lingual areas. However, the better performance during the 1-day-retrieval elicited additional bilateral cingular (BA 24, 31) and bilateral anterior frontal (BA 10, 46) activations and less activation peaks in BA 37 than in the analysis of covariance where performance was partialled out.

For the contrast of 1 month versus 1 day, we found no significant activations, neither with nor without covariate.

Correlations (n = 20).

Brain activations during retrieval of word pairs learned with 6 runs were correlated with either temporally lagged (different delay) or concurrent (same delay) retrieval performance (% correctly Remembered - % falsely Remembered) or reaction time.

The individual activity in the bilateral hippocampi (fig. 4), the right perirhinal cortex, the left amygdala, the left anterior cingulate, and bilateral retrosplenial cortices during the 1-day-retrieval predicted the individual decrease in retrieval performance between the 1-day-lag and the 1-month-lag (table 2). This means the more activated those areas during the 1-day-retrieval, the higher was the difference in retrieval performance day – month.

Similar MTL activations (at $p = 0.005$), including the right hippocampus (26, -17, -19; $t = 3.5$ and 34, -22, -16; $t = 3.6$), the left hippocampus (-26, -22, -11; $t = 3.5$), and the left amygdala (-28, -7, -22; $t = 3.0$ and -26, -3, -15; $t = 3.5$) during the 1-day-retrieval predicted the number of word pairs retrieved after 1 month. This means the higher the signal during the 1-day-retrieval, the lower the 1-month-retrieval-performance.

No positive relationship of predictive activity during the 1-day-retrieval and retrieval performance after 1 month could be observed.

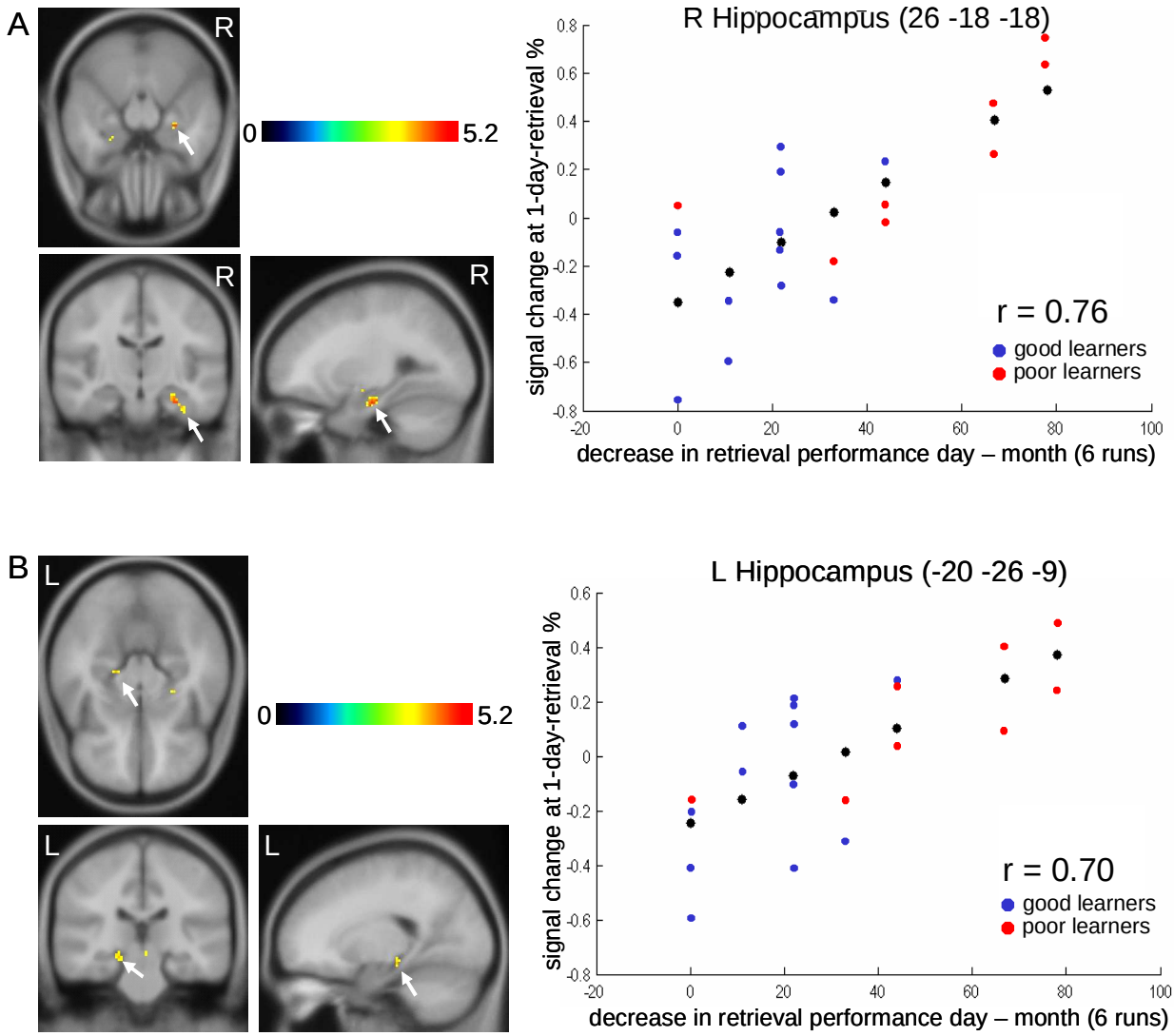

Figure 4. Correlation of fMRI signal at the day-retrieval vs. baseline with decrease of retrieval performance from day to month. Significance was yielded in the right (A; r = 0.76) and left (B; r = 0.70) hippocampal formation at the indicated Talairach coordinate positions. Large black dots indicate the regression line, small dots stand for the individual subjects, blue dots for good learners, red dots for poor learners. The corresponding T-maps (SPM2b) on the left hand are superimposed on the canonical MNI brain. Correlation strength is given in color-coded T-values. R, right side of brain; L, left side of the brain.

Regarding correlation of retrieval related activity with its concurrent performance (% correctly Remembered - % falsely Remembered), activations in the right inferior frontal gyrus (BA 47), the left fusiform gyrus (BA 20, 36), and right superior parietal lobule (BA 7) positively correlated with the 1-month-retrieval-performance whereas activations in the right inferior temporal gyrus (BA 37), the superior parietal lobule (BA 7), and the middle occipital gyrus (BA 19) positively correlated with 1-day-retrieval-performance.

Negative correlations of brain activity with retrieval performance at the 1-day-lag (the higher the activity, the worse performance) were observed in left inferior and left middle temporal gyri (BA 21, 37), in bilateral fusiform (BA 18, 19) and in left inferior frontal (BA 10, 47) areas. No negative correlations of brain activity and retrieval performance emerged at the 1-month-lag.

Correlations of reaction times with the concurrent fMRI data showed that the longer a participant took to give a correct answer during the 1-day-retrieval, the more activated were the left hippocampus (-32, -24, -9; t = 4.3), the left inferior and middle frontal gyri (BA 10, 44, 46, 47) and the right middle frontal (BA 10), and orbital (BA 11) gyri. No significant negative correlations were observed during the 1-month-retrieval.

Table 2. Activations during the 1-day-retrieval predict the decrease in retrieval performance (1-day- retrieval performance – 1-month retrieval performance) after one month.

Region of activation	activation after one day predicts decrease in performance after one month				
	x	y	z	BA	T
R. hippocampus	26	-18	-18		5.0
R. perirhinal c.	32	-15	-23		4.2
L. hippocampus	-20	-26	-9		4.2
L. Amygdala.	-28	-7	-23		5.2
R. retrosplenial c.	6	-52	6	26	4.5
L. retrosplenial c.	-6	-52	6	26	4
R. fusiform g.	16	-43	-5	37	4.1
L. precuneus	-14	-63	23	31	4.0
L. middle frontal g.	-30	44	24	46	4.5
R. orbital g.	6	60	-10	11	3.9
L. anterior cingulate	-8	43	-2	32	3.9

R, right ; L, left ; c, cortex; g, gyrus; BA, Brodmann area;
x, y, z, Talairach coordinates;

DISCUSSION

In our study, we compared cerebral activity during retrieval of word pairs after 1 day versus retrieval of word pairs after 1 month. We used a prospective design which allowed the control of critical elements retrospective designs generally lack: the strategy and number of runs during encoding and the amount and quality of retrieval.

Participants who reported rehearsal between study and test were excluded from the analysis. Retrieval was measured with a Remember/Know procedure (Tulving 1991), and the % correctly Remembered word pairs minus the % falsely Remembered word pairs were taken as retrieval performance. This strict behavioral measure made sure that detailed episodic retrieval with a strong hippocampal involvement and not retrieval based on familiarity feelings with low hippocampal involvement was measured (Henson, Rugg, Shallice, Josephs, & Dolan, 1999; Yonelinas et al., 2002; Davachi, Mitchell, & Wagner, 2003).

We compared the two time lags in a group of 12 good learners and in a group of 8 poor learners under three conditions: empirically equalized retrieval performance (analysis of covariance) but different learning procedures, statistically controlled retrieval performance and equal learning procedures and different retrieval performances but equal learning procedures. Further, the relationship between fMRI signal increase and corresponding retrieval performance and reaction time and also the predictive value of signal increase during the 1-day-retrieval for the retrieval performance after 1 month was assessed using the whole sample of 20 participants.

The time lag analysis in the good learners group revealed one clear main result that could be observed under all three conditions: The medial temporal lobe was more strongly activated during the 1-month-retrieval than the 1-day-retrieval. In the comparison of empirically equalized performance this effect was observed in bilateral parahippocampal areas, in the comparison with equal learning procedure and statistically equalized performance bilateral hippocampi showed higher activations for the 1-month- than the 1-day-lag and when performance was not equalized, still a left hippocampal effect could be observed. Together with the MTL activations, left fusiform (BA 18), left frontal (BA 47) and left temporal (BA 22, 38) areas, a network which subserves the processing and retrieval of visual-verbal and semantic information, showed stronger activations during the 1-month-retrieval than the 1-day-retrieval. In the small group of poor learners a somewhat reversed pattern could be observed. MTL and neocortical temporal, parietal and frontal activations were more pronounced at the 1-day-lag than the 1-month-lag. A reason for this might be that the poor learners showed such a high decrease in performance from retrieval after 1 day to 1 month that at the 1-month-lag too few retrieval events took place to produce retrieval associated activations.

The other main result was revealed by the correlation analysis. The results demonstrated that the more strongly activated bilateral hippocampi and neocortical areas that are commonly associated with memory retrieval, such as the left anterior cingulate, and bilateral retrosplenial cortices during the 1-day-retrieval, the greater the decrease in retrieval performance from 1 day to 1 month. Although we found the strongest effect for the decrease in the performance difference between 1 day and 1 month, the same direction of relationship was apparent at a more liberal threshold of $p = 0.005$ in the correlation of the fMRI signal during the 1-day-retrieval and retrieval performance after 1 month. Importantly, those participants with a high decrease in retrieval performance from the 1-day- to the 1-month-retrieval are not equal to those with a high starting level during the 1-day-retrieval (Fig. 3). This excludes the possibility that the positive correlation of the fMRI signal with decrease in performance emerged due to a disproportionately high performance and thus stronger activity in the group with the marked decline from the 1-day to the 1-month-retrieval.

Further, there was a positive correlation of reaction time with corresponding cerebral activity in the left hippocampus, and left and right prefrontal areas. The longer a participant needed to remember a presented word pair after 1 day, the higher activations emerged in these areas. Surprisingly, we found no positive relationship between retrieval-related MTL activations and concurrent retrieval performance (see Bosshardt et al. in press).

Our data show that the hippocampus appears to keep its important role in the retention of word pairs at least up to 1 month after learning – much in contrast to findings in animals where memory consolidation related decrease in metabolic activity in the MTL could already be observed 25 days after learning (Bontempi et al. 1999). To our knowledge, this is the first prospective neuroimaging study that demonstrates MTL activations during delayed episodic retrieval after a study-test interval of 1 month. Our finding is in line with studies of autobiographical memory reporting hippocampal activations during the retrieval of even much older episodic information (reviewed by Maguire, 2001). Unfortunately, none of the autobiographical studies investigated the hippocampal involvement in memory retrieval over such a short time period. Importantly, our study does not only demonstrate a continuous

involvement of the hippocampal formation over one month but even an increase in retrieval-related structures at the 1-month-lag. Regarding the two theories of memory consolidation out data favor the view that the hippocampus is continuously involved in the storage and retrieval of episodic information (Nadel & Moscovitch, 1997). Although an increase in fMRI signal during remote retrieval has never been explicitly discussed in the literature, the increase in the MTL at the 1-month-retrieval versus the 1-day-retrieval may be evidence for the proliferation and spreading of memory traces during the process of consolidation as proposed by the MTT. This multiple representation of the memories in the MTL serves a better retaining and retrieval of the learned episode and counteracts forgetting. However, since the investigated time period is rather short, the possibility that the spatial reorganization suggested by the standard model (Squire & Alvarez, 1995) still occurs at later stages of memory consolidation has to be taken into account theoretically.

The question arises whether there are alternative explanations for the enhanced activation after 1 month versus 1 day. Interestingly, Maguire et al. (2001) investigated whether recollecting very recent to remote memories was associated with parametric changes in brain activation and observed a non-significant trend for an increase of hippocampal responses with memory age. Unfortunately, the increase in hippocampal activity – albeit not significant – during remote versus recent memory recollection was not discussed.

To explain an increase in hippocampal activation during memory retrieval, two different mechanisms have to be taken into account. First, increased activity is related to an increase in retrieval events. There are numerous studies which showed a positive relationship between retrieval success and BOLD signal increase in the hippocampal formation (Grasby, Frith, Friston, Frackowiak, & Dolan, 1993; Nyberg, McIntosh, Houle, Nilsson, & Tulving, 1996; Gur et al., 1997; Tulving, Habib, Nyberg, Lepage, & McIntosh, 1999; Cameron, Yashar, Wilson, & Fried, 2001). However, since performance is equalized both empirically and statistically, our increase in MTL activity cannot be explained by increased retrieval success.

The second mechanism that has been postulated is that an increase in spatial extent and magnitude of activations is determined by increasing task demands in a variety of cognitive tasks (Just, Carpenter, Keller, Eddy, & Thulborn, 1996; Braver et al., 1997; Jonides et al., 1997; Carpenter, Just, Keller, Eddy, & Thulborn, 1999;

Honey, Bullmore, & Sharma, 2000). Because of their immature cognition, task demands are naturally increased for children, when compared to young adults. As a consequence, increased activity in structures that mediate task performance would be expected. Indeed, Luna et al. (2001) and Durston et al. (2002) both reported stronger activations of prefrontal and parietal networks during tasks of inhibitory control in children or adolescents compared to young adults. The increased activations were interpreted in terms of an attempt to compensate for an inefficient network by means of an increased recruitment of the brain regions that mediate task performance. The inefficient use of the fronto-parietal network in children may reflect yet immature brain systems. Alternatively, increased activity can also be an indication for already deficient functions due to pathologic aging processes. In this case, increased compensatory activity was not only reported for neocortical networks but also for the hippocampus. In fact, Bookheimer et al. (2000) reported greater magnitude and extent of brain activation in the hippocampus, parietal cortex and prefrontal cortex during a memory task in healthy participants who were genetically at risk for Alzheimer's disease versus control participants without the risk factor. Since both groups had equal performances, Bookheimer et al. (2000) interpreted the signal increase as compensatory cognitive work to bring memory-related performance to a normal level. If we assumed that the retrieval of word pairs or sentences learned 1 month before needed more cognitive resources than the retrieval of an equal number of word pairs learned the day before, this second mechanism could account for the increase found after 1 month.

This assumption is supported by the results of the correlation analyses.

We observed that the bilateral hippocampal signal increase during retrieval after 1 day versus baseline was highest for those participants who showed a high decrease in retrieval performance from the 1-day-lag to the 1-month-lag. To interpret this correlation pattern we assumed that those participants who showed a large decrease in performance after 1 month also experienced a higher cognitive demand at the 1-day-lag already. As a result, this group of "high-forgetters" that corresponds mainly to the group of poor learners had to rely more on the relevant networks to perform as successfully as the good learners at the 1-day-lag. So, the higher fMRI signal can be explained by an increased recruitment of the regions crucial for the task. Importantly, this increased recruitment does not serve directly the retrieval of correct word pairs because no positive correlation between retrieval success and MTL signal could be

observed. Rather, it appears to be related to additional cognitive work subserving retrieval and rejection of false answers. This hypothesis is supported by the finding that the higher activations in the left hippocampus and bilateral frontal areas during the retrieval at the 1-day-lag are associated with longer reaction times.
At the 1-month-lag however, poor learners are not anymore able to compensate for their low memory capacity. The cognitive demand gets too high and thus the inability to recruit the crucial structures efficiently finds expression also in the decay of retrieval performance.
The good learners retrieve the word pairs they learned the day before easily. Only after a month's time, they will show similar difficulties with retrieval as the poor learners had already after 1 day. So the increased signal after 1 month could alternatively be interpreted in the context of increased computational demands to retrieve an equal amount of information as after 1 day.

Increased activations at the month-retrieval versus the day-retrieval in the good learners and vice versa in the poor learners were also observed in neocortical regions that are part of the verbal memory retrieval network, such as left anterior temporal, inferior frontal and fusiform areas (table 1). Further, apart from MTL areas, activations in the left anterior cingulate and bilateral retrosplenial cortices during the 1-day-retrieval predicted the performance decrease from 1 day to 1 month. Therefore, it seems that increased task demands not only result in a greater recruitment of medial temporal structures but of retrieval-related networks in general.

Surprisingly, we did not find any positive relationship between MTL activations and retrieval performance as we did in our previous study (Bosshardt et al., 2004). A critical difference between the two studies is that the level of retrieval performance is significantly higher in the present study. Therefore, one might imagine that the level of performance determines the nature of relationship between hippocampal activity and retrieval success.

In conclusion, our study demonstrated that after 1 month of consolidation the hippocampal involvement in memory retrieval does not decline. On the contrary, we even observed an increase in both hippocampal and neocortical areas during retrieval after 1 month versus 1 day. This increase may reflect a proliferation and

spreading of multiple related memory traces as predicted by the MTT (Nadel and Moscovitch, 1997). Alternatively, it could be interpreted in terms of a greater reliance on retrieval-relevant structures when task demands increase, i.e. during the retrieval of information that was stored 1 month ago.

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4. GENERAL DISCUSSION

We compared retrieval-related cerebral activity after two different consolidation periods with a prospective design. We were interested in whether an fMRI signal enhancement could be observed after the initial 24 hours of consolidation and further enhancement after 1 month following this 24 hours period.
In both studies, we controlled the variables that are confounding the research of many studies of memory consolidation, such as learning procedure, episodic quality of retrieval, and rehearsal between study and test. Incidental learning of word-word associations ensured that the learning material was processed similarly by all participants. The Remember/Know procedure (Tulving, 1985) specified whether the retrieval of word pairs was truly episodic or merely semantic in nature. Furthermore, none of the participants taken into data analysis indicated having rehearsed the learning material in the time between study and test.

4.1 The first study

The first study compared brain activations during the retrieval of word-word associations 10 minutes versus 24 hours after learning. The outcome was an increase in retrieval-related anterior hippocampal activity at the 24-hour- versus the 10-minute-lag and a strengthening of the relation between hippocampal activity and retrieval success over 24 hours. According to the literature the left anterior hippocampus as opposed to posterior parts is especially involved in relational processing of multiple stimuli, including the semantic encoding of word-word associations (Dolan and Fletcher, 1997; Henke *et al*., 1999). Relational processes are also hypothesized to produce similar anterior hippocampal activations during retrieval (Schacter and Wagner, 1999). An increase in the anterior hippocampus during retrieval may be interpreted as a stabilization of memory traces over the first 24 hours of memory consolidation. In the neocortex, there was a greater demand for left prefrontal semantic areas, the left precuneus and bilateral inferior parietal areas at the 10-minute-lag. Conversely, the reliance on areas of episodic memory retrieval mode (REMO) (BA 10) which stand for the control and monitoring of incoming online information as possible retrieval cues (Tulving et al., 1994; Lepage et al., 2000;) was greater after 24 hours. These neocortical results implied that stronger semantic

representations were needed at a time when memory traces were still fragile. After 24 hours however, the system of episodic memory retrieval mode (REMO) had strengthened its control over retrieval, perhaps rendering the semantic representations more focussed.

In conclusion, the results of the first study support our hypotheses. The finding of an increase in hippocampal activity after 24 hours may be regarded as a functional neuroimaging correlate of the neurobiological findings of an increase in hippocampal synaptic connectivity (O'Malley et al. 2000; Kandel, 2001; Leuner *et al*., 2003) taking place within the first 24 hours of memory consolidation.

4.2 The second study

The second study investigated consolidation effects over 1 month with a similar verbal associative learning paradigm as was used in the first study. We compared retrieval-related cerebral activity 1 day versus 1 month following learning in a group of 12 good learners and a group of 8 poor learners. Good learners remembered an equal amount of word pairs at the two delay times. Poor learners remembered significantly less word pairs than good learners at the 1-month-retrieval but not at the 1-day-retrieval.

In the good learners, the retrieval after 1 month yielded higher MTL activations than the retrieval after 1 day, indicating that the MTL does not diminish its involvement in memory retrieval after 1 month of consolidation. Equally, left neocortical areas subserving the processing and retrieval of visual-verbal and semantic information, including inferior frontal (BA 47), temporal (BA 22, 38) and fusiform (BA 18) areas exhibited also enhanced activations at the 1-month lag versus the 1-day-lag. Conversely, poor learners exhibited a decrease in MTL and retrieval-associated neocortical activations over time, probably reflecting their dramatic decrease in number of retrieved word pairs over 1 month.

Moreover, over the whole group the amount of forgetting over 1 month was predicted by the retrieval-related activity in the MTL at the 1-day-lag, i.e. those participants who suffered from higher forgetting rates over the 1-month-interval had higher increases in MTL activity during the 1-day-retrieval already.

The results of the second study do only partially support our hypotheses. First, in the good learners who performed equally at the two time lags, we were not able to detect a consolidation-related decrease in hippocampal activity as was hypothesized on grounds of findings in animals. Conversely, we even found an increase in MTL and neocortical areas. The decrease in MTL activity observed in the group of poor learners may rather be related to the marked decrease in performance than to any consolidation processes. Second, hippocampal activity at the 1-day-lag did indeed predict how good the word pairs were retained over 1 month. However, we expected that an increase in activity will have a positive effect on retention because we assumed that stronger activity means a better integration of learned information in the memory network. In contrast, we found that higher activity at the 1-day-lag is related to higher forgetting rates. A possible explanation for this rather unexpected finding will be discussed at a later stage (see 4.5).

4.3 Short term changes – the dynamic nature of memory

Most importantly, the present results clearly demonstrate that fMRI is able to detect changes in retrieval-related MTL brain activity already after short consolidation times, such as 1 day or 1 month. Importantly, the changes cannot simply be put down to differences in the amount of truly episodically retrieved word pairs because we controlled for the number of remembered word pairs in both studies. The second study moreover controlled for the learning procedure, i.e. learning instructions and number of learning runs. Our findings are the first to demonstrate a retrieval-related signal change after such short consolidation periods. The only other study that aimed to investigate consolidation with a prospective design (Stark and Squire, 2000) found no signal changes during the retrieval one week versus one half hour following learning. The reason for the discrepancy of their results and ours may be the different learning paradigms. Whereas we used incidental verbal associative learning and emphasized on true episodic retrieval which was controlled for by means of the Remember/Know paradigm, Stark and Squire (2000) used a figural recognition task and did not control for the quality of the retrieval.

Most other neuroimaging studies on consolidation investigated autobiographical memory over time periods of years or even decades (Nadel et al., 2000; Haist, 2001;

Maguire et al., 2001; Niki and Luo, 2002; Tsukiura et al., 2002; Piefke et al., 2003). The results of these studies are controversial with some studies pointing to a decrease of MTL involvement in retrieval of remote memories over time and others demonstrating that recent and remote retrieval are associated with equal MTL activity measured by fMRI or PET. The equivocal findings may result from different methods that were used to assess autobiographical memories (Maguire, 2001). The used time frames were probably chosen on grounds of studies on retrograde amnesia in patients with MTL lesions which implied that memory retrieval requires the MTL for such long times before it gets eventually independent of these structures. However, from a bio-logical view it is not conceivable that events are stored for as long as a life time before such reorganization takes place (Nadel and Moscovitch, 1997).

Instead, there is the hypothesis of a dynamic memory system that undergoes changes from the moment of acquisition of new material probably until the material has to be retrieved many years later. Specifically, the multiple trace theory proposes that over time multiple memory traces are formed and that this process contributes to the stability of memories (Nadel and Land, 2000). The formation of multiple traces is not a simple addition of new traces but the old traces are subject to change, i.e. some aspects of a memory might be strengthened, others weakened. Our data support the idea of a dynamic memory insofar as they demonstrate changes in the activity of MTL and neocortical structures during retrieval already after 1 day and again after 1 month.

In cellular consolidation research, this idea of dynamic memory storage including consolidation and reconsolidation has long been established (Nader *et al*., 2000b). The cellular mechanisms of consolidation are mechanisms of plasticity such as the strengthening of existing synapses, the formation of new synaptic spines and eventually the growth of new connections (Milner et al. 1998;. It is possible that our fMRI signal changes represent those mechanisms of plasticity provided that these mechanisms involve changes in glutamatergic synaptic activity, which is the process that can be measured with fMRI (Magistretti and Pellerin, 1999; Logothetis et al., 2001; Raichle, 2001; Bonvento et al., 2002; Lauritzen and Gold, 2003; Zonta et al., 2003). Our findings of activity changes early after learning are particularly important because they are a first attempt to bridge the gap between cellular and neurocognitive consolidation research.

4.4 Systems consolidation

In systems consolidation literature, there is still no consensus about whether the hippocampus has a time-limited role in memory retrieval or whether it is involved in retrieval no matter of the memory age. The former is proposed by the standard model of consolidation (Squire and Alvarez, 1995) and is supported by neuroimaging studies that found a retrieval related decrease in MTL activity from recent to remote memory retrieval. The multiple trace theory (MTT) (Nadel and Moscovitch, 1997) holds the latter opinion and is so far primarily confirmed by neuroimaging studies that found no activity differences between the retrieval of recent versus remote memories. Certainly, we are interested in whether our imaging findings may contribute to this debate. The advantage of both our studies, and especially the second one, is that the possible confounding variables that may have been the reason for the disagreeing results so far were largely controlled for.

Our second study shows an increase in MTL activity at the 1-month- versus the 1-day-retrieval.

None of the consolidation theories has ever discussed an increase in fMRI signal in the MTL over time. The classic view (Squire and Alvarez, 1995) assumes that memory retrieval becomes progressively independent of the MTL which would be associated with a decrease in the associated fMRI signal. Yet, the theory does not make predictions about how this independency is achieved. It is not clear, whether there should be a linear decrease of involvement and associated fMRI signal from the moment of storage until the reorganization is complete or whether the process follows a different pattern in which the decrease would occur rather rapid at a given time.

The defendants of the MTT (Nadel and Moscovitch, 1997; Nadel et al., 2000) predict that there is no decrease in memory retrieval-related MTL activity over time, at least as long as the episodic quality of recent and remote retrieval is equal. Since we controlled for episodic quality empirically and statistically, the increases in our studies cannot be explained by a higher episodic quality of the remote memories. Importantly, at the core of the MTT stands the proliferation of memory traces over time. Although the authors never make any suggestions about the structural underpinnings of these abstract memory traces it is imaginable that the proliferation of the traces would be associated with an increase in synaptic connectivity and

consequently with synaptic activity during retrieval. If this were the case, we could speculate that this proliferation indeed could result in an increase in fMRI signal, given that the BOLD signal is an indirect measure for glutamatergic synaptic activity (Magistretti and Pellerin, 1999; Logothetis et al., 2001; Raichle, 2001; Bonvento et al., 2002; Lauritzen and Gold, 2003; Zonta et al., 2003). Along this line of argumentation, our result of an increased MTL activity during the 1-month versus the 1-day-retrieval is clearly compatible with the MTT (Nadel and Moscovitch, 1997) and rather speaks against the traditional model.

According to the MTT, our data suggest that already during the first month of consolidation, new traces are formed and distributed over the memory network. This proliferation and spreading of related memory traces allows for retrieval of the learned information after one month that is equal to the retrieval after one day with respect to both the number of retrieved items and the episodic quality of the memories. Also, the multiple representations of the memories make them less prone to forgetting.

However, in the tradtitional view of memory consolidation very long time intervals are proposed to be necessary for the processes that eventually detach memory retrieval from the hippocampal formation (Stark and Squire, 2000). Theoretically, one could argue that after a primary increase of hippocampal engagement in memory retrieval a spatial reorganisation could still take place in the long run. Although this scenario is rather improbable, the standard model of memory consolidation can only definitively be refuted when controlled studies with longer time intervals between study and test have shown that remote memory retrieval is still associated with activity in the medial temporal lobe.

4.5 Prediction of memory retrieval after one month

A rather unexpected finding in the second study was revealed by the correlation analysis over the whole sample of good plus poor learners. The strength of the fMRI signal in bilateral hippocampi during the 1-day-retrieval predicted the amount of forgotten word pairs after 1 month, i.e. the stronger the signal, the higher was the decrease in performance from the 1-day- to the 1-month-retrieval. This finding is

contradictory to what we expected. We hypothesized that an increase in activity would have a positive effect on later retrieval due to a stronger integration of learned information in the memory network. But our data imply a different mechanism. At first sight it appears that this effect could be a baseline artefact, meaning that those participants who show a high decrease in performance between the two time lags may have started at a much higher level than the others, suggesting that the decrease was a relative one and not one that resulted in poor performance after one month. But this was not the case. There was no difference in performance at the 1-day-lag between those who retained the material properly and those who showed a marked decrease. Also, the decrease led to a very poor performance at the 1-month-lag, making this group with a high decrease mostly congruent with the group of poor learners (see Fig 3b, p.88). Thus, it seems that at the 1-day-lag good learners activated bilateral hippocampi less strongly than poor learners and nevertheless managed to retain a high amount of word pairs over the 1-month-interval.

A possible explanation for this contra-intuitive finding can be found in studies that demonstrate that an increase in fMRI activity may also be associated with an increase in computational demand. This means that the more efficiently a task is dealt with, the less cognitive resources and accordingly less neural recruitment is necessary. Examples for such an inverse relationship are given in various cognitive areas, such as sentence comprehension (Just et al. 1996), visuospatial processing (Carpenter, et al. 1999), and executive functions (Luna et al. 2001).

Importantly, an increased neural engagement as a result of inefficient cognitive work was also demonstrated for the hippocampal formation. Bookheimer et al. (2000) suggested that hippocampal structures were recruited as a compensation for an otherwise poor retrieval performance in healthy older adults who carry a risk gene for Alzheimer's disease. In their study, both the extent and magnitude of brain activations during memory tasks in hippocampal, prefrontal and parietal areas were greater for the carriers of the risk gene than for the non-carriers of this risk gene. Crucially, the genetic risk participants did not differ in memory performance from the control participants. Bookheimer et al. (2000) interpreted the observed increases in terms of an augmentation of the brain's processing capacity to meet increased task demands in subjects at risk. This use of additional cognitive resources might have served a compensatory strategy to achieve the same level of performance as subjects who were not at genetic risk.

Accordingly, in our study the increase in medial temporal and neocortical structures at the 1-day-lag that is associated with a high decrease in performance over one month could be interpreted as a result of such an increased neural recruitment when task demands are high. We assumed that the participants who suffered from high forgetting rates over 1 month had an overall lower memory capacity and therefore also experienced a higher cognitive demand at the 1-day-lag. Yet, this higher demand did not find expression in a decrease in retrieval performance at the 1-day-lag but only at the 1-month-lag. Nevertheless, we hypothesized that these participants subjectively experienced increased task demands resulting in activity increases in MTL and neocortical areas as an expression of an intensified recruitment of retrieval-related neural structures. This hypothesis was supported by the finding of a correlation of hippocampal activity at the 1-day-lag with concurrent reaction time. The longer a participant needed to give his or her answer, the stronger the hippocampus was activated. This implies that this increase in activity is related to processes that do not result in an increased retrieval of correct word pairs but rather may assist retrieval indirectly by subserving retrieval and rejection of inappropriate answers until the correct answer has been found.

In summary, the positive correlation of bilateral hippocampal activity at the 1-day-lag with a decrease of performance over the 1-month-interval may be explained by a compensatory recruitment of neural structures in poor learners. At the 1-day-lag, poor learners remembered as many word pairs as the good learners but due to their lower memory capacity needed probably more neural resources to realize the same performance as the good learners. Conversely, the good learners who retained many word pairs over one month may have had more efficient retrieval strategies at the 1-day-lag than the poor learners resulting in a less strongly activated retrieval network.

One could speculate that such an increase in signal due to a compensatory neural recruitment could also have contributed to the activity increase in neocortical and MTL structures after 1 month observed in the good learners. It is imaginable that subjective task demands increase with an increasing interval between study and test. A reason for this could be an increasing number of interferences. Thus, the group of good learners might have needed more cognitive resources to bring performance during the 1-month-retrieval to an equal level as during the 1-day-retrieval. Consequently, retrieval-related brain structures would have been activated more

strongly and more extensively than at the 1-day-lag. Possibly, this mechanism added to the increase that resulted from consolidation processes. One could even speculate further and suggest that the consolidation processes that led to a proliferation and distribution of related memory traces support this enhanced recruitment of retrieval-related neural structures and allow for a successful retrieval of otherwise irretrievable information.

5. CONCLUSIONS

We have demonstrated with a prospective design allowing the control of learning, retrieval quality and rehearsal between study and test that already after short consolidation periods, such as 1 day and 1 month increases in the retrieval-related fRMI signal can be observed in the MTL. The increases after 1 day are hypothesized to reflect consolidation-related cellular changes, such as an increase in synaptic activity or connectivity. The increase after 1 month may be evidence for a proliferation and spreading of memory traces as proposed by the multiple trace theory (MTT, Nadel and Moscovitch, 1997). Additionally, it may be related to an enhanced recruitment of a memory retrieval network including the MTL and neocortical areas as a consequence of an increased cognitive demand during the retrieval of remote versus recent memories.

Our results are the first to show changes in the fMRI signal during the retrieval of consolidated memories after such short delay times. Further research is needed to provide a clearer picture about the functional changes in the memory network during the first weeks of memory consolidation.

Regarding systems consolidation theory, our data preferentially support the MTT because the signal increase after one month could be interpreted in terms of reflecting multiple related memory traces. However, the spatial reorganization as it is proposed by the standard model can take place at a time that is beyond the time period we examined and therefore the traditional view cannot be refuted until evidence of increased activities have been found for longer intervals between study and test.

An open question that is raised by the study concerns the determinants that result in a proliferation of memory traces and consequently lead to a better retention of information over time. In other words, what differs the good from the poor learners? Although memory performance at the 1-day-retrieval is equal, the two groups differ in their way they engage the retrieval network. As the correlation of memory performance at the 1-month-lag with the signal during the 1-day-retrieval demonstrated, poor learners more strongly relied on the retrieval network for an equal performance as achieved by the good learners. This finding implies that good and poor learners may have different memory capacities in general and not only regarding long-term retention and retrieval. More research is needed to illuminate the

differences in the various memory processes and their neural underpinnings between good and poor learners.

Our data are in line with recent articles suggesting that the storage of information over the long term is far more dynamic than assumed before. Traditional consolidation theory claims that consolidation makes a memory trace permanent and insusceptible to disruption (Kandel, 2001). The theory distinguishes two sequential stages: a labile short-term memory lasting for seconds to hours is followed by a stable long-term memory lasting for days and longer. An inversion from stable to labile is not predicted by the theory. However, the findings that reactivation of an established memory returns this memory into a labile state again (Misanin, 1968) imply that a complete fixation of a memory into a stable and invulnerable trace does never occur. Conversely, the existing traces appear to be subject to permanent change. Changes can be triggered by explicit reactivation by renewed exposure to the training context or learning stimuli or, as shown by Sutherland and McNaughton (2000), reactivation can occur also spontaneously during sleep or quiet wakefulness. Psychologically, the permanent modification of existing traces has important implications. It allows an updating and refinement of memories. Updating includes the integration of new information with the existing representations. Every reactivation of a memory by re-exposure to the learning context is associated with the storage of new contextual details since two learning experiences can never be exactly the same. Therefore, reactivation is likely to include the formation of new traces as proposed by Nadel and Land (2000). These newly formed associated traces of context information are presumably episodic in nature and therefore again particularly vulnerable to disruption by hippocampal pathology. On the other hand refinement includes the reevaluation of which aspects of the memory are worth to be kept in mind. The idea that memory is not a unitary entity is also part of the MTT (Nadel and Moscovitch, 1997, Nadel and Land, 2000). They claim that various aspects of a memory may experience different fates with time. It is conceivable that as a result of the reactivation process mainly the essence of a given memory will be strengthened by representation as multiple traces whereas contextual details will be changed or get lost. Over the time and after many reactivations, this essence may become more and more detached from the episodic context and gain a semantic character. Importantly, those semantic contents may then be retrieved independently of

hippocampal function (Nadel and Moscovitch, 1997). It is established that retrograde amnesias for semantic memories are much less extensive than for episodic memories (Kopelman, 2000) indicating that only true episodic information remains dependent of hippocampal function. Thus, reactivation and reconsolidation in the long-term support the semantization of information which means that the gist of an episode, i.e. the facts of what happened, is preserved whereas perceptual and contextual details which are often negligible aspects of a memory get lost (Fig. 8).

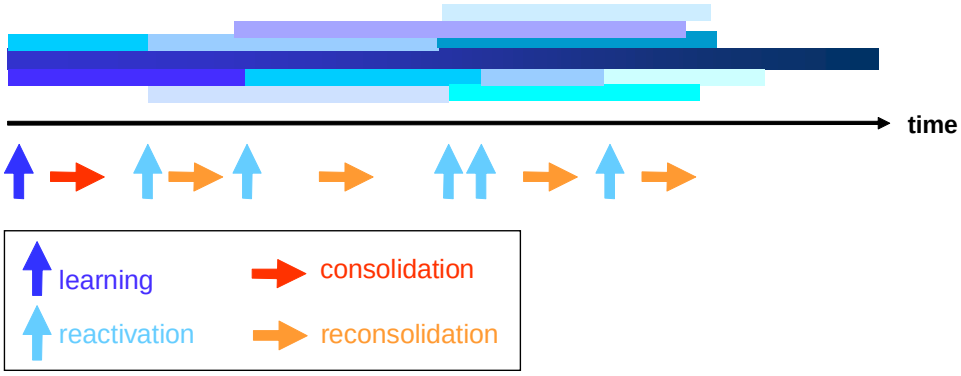

Fig. 8. Hypothetic model of the semantization process in the course of memory consolidation. Repeated consolidation and reconsolidation in the hippocampal formation supports the formation of multiple memory traces. Whereas traces of episodic details (various light blues) are changed, added and modified, the trace representing the gist of the memory (dark blue) gets strengthened. Over time the essence may be detached from the episodic context and thus be represented at neocortical sites.

It may well be that most memories that are stored for a very long time loose their episodic nature and become more semantic and fact like over time (see also Rosenbaum et al., 2001). So, the often reported graded patterns of retrograde amnesia with preserved remote memories may result from a bias in remote memories being more fact-like and free of episodic details and therefore retrievable despite MTL pathology. The same bias would explain the fact that AD patients seem to have a much better memory for remote episodes than for recent ones. This has also been claimed by Piolino et al. (2003) who reported that AD patients had more difficulty to retrieve truly episodic remote memories compared with more generic autobiographical ones without autonoetic consciousness. Episodic memories that have been semanticized over the years are less sensitive to disruption by hippocampal pathology because they are represented mainly by neocortical

structures. Conversely, very recent semantic memories seem to be dependent on the MTL since they are still contextually bound (Rosenbaum et al. 2001) and bear a high autobiographical significance (Westmacott et al. 2003).

The hypothesis of a semantization of episodic information over time is supported by the everyday experience of the difficulty to retrieve true episodic information of events that happened many years ago. The memories are faint and lack the colorfulness and richness of very recent memories. Thus, it seems that the process of semantization is an important aspect of consolidation because it looks as if it protected memories from being forgotten either by a too long interval between learning and retrieval or by damage to MTL structures.

An extensive testing of the semantization hypothesis in healthy participants and patients with hippocampal damage could be a topic for further research. The ratio of semantic and episodic information should be varied in a parametric design and the associated medial temporal involvement assessed over time.

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